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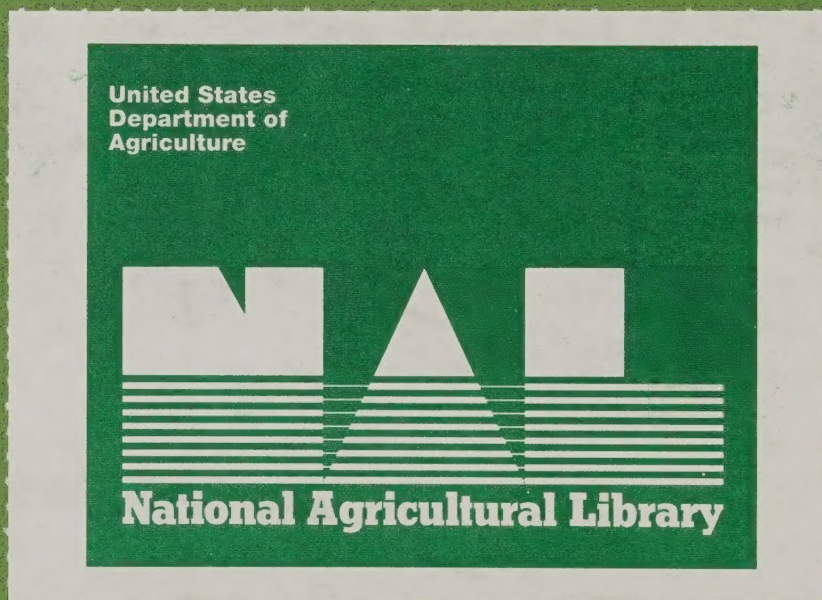
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USDA-ARS Action Plan for Fruit Flies Research

Faust, Robert M. and James R. Coppedge,
eds. 1992. USDA-ARS Action Plan for Fruit
Flies Research. U.S. Department of
Agriculture, Agricultural Research
Service, 190 pp.

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USDA-ARS ACTION PLAN FOR FRUIT FLIES RESEARCH

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PREFACE

This National Action Plan details the proposed ARS fundamental and applied fruit fly research program in cooperation with universities and other state and federal agencies. A cohesive team effort is being mounted to help solve specific national problems related to fruit flies. This will require detailed formulation of an action program that clearly defines and states program goals and objectives, identifies each project's relevance and role, identifies activities to reach the objectives, establishes time frames needed to reach objectives, and provides a basis for full participation of ARS scientists and cooperators in planning the program. The action plan is designed to meet this need and to evolve into a comprehensive national plan composed of an aggregated strategic and operational plan for fruit fly research. As it evolves, this comprehensive plan will provide (a) focus and programmatic stability, (b) a basis for monitoring and evaluating program progress, (c) a basis for developing budget estimates and allocating resources, (d) responsiveness to the technology and problem-solving needs of state and federal action agencies, (e) identification of technology transfer opportunities, and most importantly (f) development of team players and teamwork. The action plan will provide an important foundation for program strengthening and expansion, coordination, decision making, and implementation by the ARS National Program Staff.

The primary aim of the national fruit fly program is to provide the necessary research--through a cohesive team effort--that will lead to environmentally and publicly acceptable, safe technologies for (a) detection and delimitation, (b) exclusion, (c) control and eradication, and (d) fundamental biology. The technologies developed will support the implementation of state and federal action and regulatory programs. ARS is supportive of these state and federal goals; i.e., to reduce the threats to the United States from exotic fruit fly pests by preventing their introduction and establishment, and to reduce the negative impacts of established fruit fly pests. The plan is designed to be dynamic yet responsive to the needs and priorities of our stakeholders. Thus, progress in reaching the goals of the plan will be reviewed on an annual basis. As the program progresses, participants will play a significant role in redefining essential activities when necessary, in eliminating some proposed activities that may result from the inherent uncertainties of research, and in assigning appropriate remaining activities or selecting new activities to achieve goals. This is the dynamic nature of the plan.

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warranty of the product by the U. S. Department of Agriculture or an endorsement by the Department over other products not mentioned.

The National Program Staff expresses its gratitude and appreciation to all conference attendees for participating in the organization of the conference and in formulating this comprehensive action plan. The conference was highly productive, positive, and met the objectives originally set forth. We are especially indebted to the representatives from APHIS, universities, state and other federal agencies, and the representatives from industry for their valuable interactions and contributions.

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EXECUTIVE SUMMARY

Tephritid fruit flies pose a major threat to the production and marketing of fruit within the United States and throughout the world. Four fruit flies, Mediterranean fruit fly, Malaysian fruit fly, melon fly and the Oriental fruit fly are residents of Hawaii, the Mexican fruit fly is a sporadic invader of Texas and California, while the Caribbean fruit fly is firmly established in Florida. The greatest threat these flies pose are as invaders of the tropical and subtropical fruit production areas of the U.S. Although they would have some affect on fruit production, the greatest problem exists with quarantine restrictions associated with distribution of these fruits within the U.S. and to foreign countries. Broad social goals of fruit fly research are similar to other areas of agricultural production efficiency research, that is, "to promote continuing efficiency in the use of agricultural resources in order to assure that an adequate supplies of agricultural products are produced at continuously lower relative cost and to develop knowledge to enable farmers to improve the returns from farming as a business by use of improved technologies for pest control."

The Action Plan is the result of joint efforts by ARS and university scientists, by federal and state action agencies and by industry. The USDA-ARS Action Plan for Fruit Fly Research contained herein is divided into four main sections and their accompanying subsections: (a) Detection and Delimitation, (b) Exclusion, (c) Control and Eradication and (d) Fundamental Biology. Also included are several appendices that list needs for research suggested by industry and action and regulatory agencies. Each subsection contains a background statement, description of needs, work plans for FY 92 by investigator(s), objective of work significance, justification, constraints, selected references and a listing of co-investigators/cooperators. Each objective is coded to the section and subsection topics within the Action Plan. Although each research activity is only described once in this document, reference to it may appear as "Also see" under other topics of the outline. The link between each research activity and the needs expressed by action and regulatory agencies and by industry representatives can be found in the codes under the Justification; the lists of these coded needs are contained in the appendices.

The Workshop was held at the Airport Marriott in San Francisco from July 29 to August 2, 1991. The work plan, that was arbitrarily divided into sections on Detection, Exclusion and Control/ Eradication, was discussed in detail in breakout sessions. Emphasis was placed on directing research objectives toward those needs expressed by Regulatory and Action Agencies and Industry.

Significant research needs toward which research objectives will be directed include:

1. Development of more powerful detection tools such as sex and food attractants and traps that are more efficient, longer lasting and easier to service.
2. Development of a systems approach for fruit fly suppression and management to expand fly-free zones in the U.S. and Latin America.
3. Development of new commodity treatments such as irradiation, controlled atmospheres, new fumigants, and artificial films such as shrink wrap.
4. Development of substitutes for malathion in bait sprays used for eradication.
5. Development of male and female annihilation techniques that would preclude the use of bait sprays.
6. Development of biological control aspects that would suppress populations in the U.S. and in foreign countries, thus reducing the probability of fruit fly larvae in distributed fruit.

As a result of the assembly of ARS fruit fly scientists, many cooperative alliances were formed where there were duplications of efforts or where undue competition existed. In addition, it became evident to other agencies and to the fruit industry that ARS is serious about a research action plan for fruit flies that will alleviate some of the problems these flies present.

OBJECTIVES AND CHARGE

The overall charge of the ARS-Wide Fruit Fly Working Conference was to develop a USDA-ARS Action Plan that would encompass fundamental and applied fruit fly research in cooperation with University collaborators and state and federal agencies. It is expected that this Action Plan will ultimately evolve into a comprehensive national fruit fly research plan. The working conference was specifically designed to provide a forum for expressing views, generating ideas and identifying gaps, needs and areas of cooperation leading to technologies for fruit fly (a) detection and delimitation, (b) exclusion and (c) control and eradication.

Specific objectives were to: 1) Foster opportunities for information dissemination and improved cooperation and coordination among ARS scientists and others having a vested interest in fundamental and applied fruit fly research; 2) Provide opportunities for input into the program plan from federal and state action and regulatory agencies, university collaborators and industry; 3) Validate the current research status and constraints to progress; 4) Identify research gaps, major areas of emphasis/de-emphasis and priority needs and to recommend activities necessary to achieve goals.

On March 13-14, 1991, an initial planning committee meeting was held in Beltsville, Maryland to discuss logistics, structure and objectives and goals for an ARS-wide working conference. As identified by the committee, the specific goals of research on fruit fly control include "development of population suppression and eradication techniques for fruit flies that can be applied to populated areas without danger to the public, without disruption of routine living conditions, without obnoxious or hazardous pesticide residues being added to the environment, to provide prompt and adequate detection of incipient infestations and to limit the extent of such infestations, to provide control without adverse effect on populations of beneficial insects, fish, wildlife and other animals and to protect the agricultural industry of the United States from the ravages of fruit fly infestations." Specific objectives for the working conference were also established during the planning meeting as detailed above.

During this planning meeting, the ARS National Program Staff appointed a steering committee of ARS and APHIS scientists who would be assigned the responsibility of drafting an action plan for ARS fruit fly research. The initial charge of this committee was to gather the necessary information for the draft plan from (a) ARS scientists conducting fundamental and applied research on fruit flies, (b) cooperating USDA and state action agencies e.g., APHIS, the California Department of Food and Agriculture, etc., (c) cooperating universities and (d) the industry including growers, suppliers, etc. Dr. Carrol Calkins, ARS, Gainesville, Florida was appointed as chairman. The ARS scientists who were working on fruit flies were asked to list their accomplishments for

the last 5 years, their plans and objectives for the next 5 years and to list the scientists within and outside ARS with whom they were cooperating. A poll was also taken of University scientists regarding their accomplishments on fruit fly research in the last 5 years and their potential cooperation in this regard with ARS scientists. An additional poll was taken of the ARS clients, i.e., industry and public agencies, regarding their viewpoints on the needs of fruit fly research.

Once the requested information was received, the committee members held follow-up discussions to gain further insight and detail into the individual ARS research projects and materials detailing needs submitted by other groups. A draft research action plan was assembled and comments solicited on the draft document.

Subsequently, the steering committee met in Albany, California, May 21-23 to discuss the draft document and its organization, the priorities and needs and the details of the process for the ARS-Wide Fruit Fly Working Conference. A final version of the draft document was sent to all participants who were encouraged to attend the Conference to be held in San Francisco, July 29-August 2, 1991. The Fruit Fly Research Action Plan contained in this document is a result of this conference. The document is particularly geared to be responsive to the technology and high priority, problem-solving needs of state and federal action agencies, as well as industry.

OVERVIEW

THE USDA-ARS ACTION PLAN FOR FRUIT FLY RESEARCH

The ARS research program on tropical tephritid fruit flies is a multi-faceted large-scale effort involving some ARS 40 SY's at 9 ARS locations. This research effort is aimed at stopping the geographical spread of these pests; providing for the safe movement in commercial agriculture of fruits and vegetables out of areas already invaded; reducing costs and damage to agriculture in those invaded areas; and, finally, developing environmentally acceptable and economically efficacious systems to eradicate established populations of fruit flies.

Threats of invasion come from Australia, Southeast Asia, Africa, the Mediterranean area, Central and South America, Mexico, and the Caribbean and Pacific Islands. In the U. S., established populations exist only in Hawaii and southern Florida but the rate of introductions of these foreign pests has increased dramatically in the last 15 years. Of necessity, numerous eradication programs have been conducted in the continental U.S. during this period. The total cost exceeds \$150 million dollars. Potential losses in terms of monetary costs and increased pesticide burden on the environment are very large. It has been estimated that establishment of the Mediterranean fruit fly in the United States would cost between \$830 million and \$1 billion dollars per year to the economy. Furthermore, its presence would seriously disrupt many of the pest management programs now in place because control efforts would necessitate extensive pesticide usage. The loss of production in dooryard crops and a concomitant increase in pesticide applications by home gardeners would increase costs and may create safety hazards.

More than a dozen species of tropical fruit fly pests have been detected invading the continental U. S. in the last five years. Some, such as the oriental fruit fly and the Mediterranean fruit fly, have required eradication programs while others have died out after failing to colonize. Successful eradication programs have also been carried out against the melon fly, the peach fly (Bactrocera zonatus) and the Mexican fruit fly. None of these invading species has become established. The only established populations of tropical pest tephritid fruit flies in the U.S. are the four species in Hawaii: the melon, Mediterranean, Malaysian and Oriental fruit flies; and two species in Florida: the Caribbean and the papaya fruit flies.

The USDA (ARS and APHIS and their predecessor agencies) has a long history of research and program application in the tropical fruit fly field going back to the second decade of this century. This effort has been marked by some signal successes spurred by innovative research and diligent application of these research results. That condition of innovation and diligent application is still evident in the on-going ARS research programs and in the APHIS fruit fly efforts. The ARS research

effort is tied closely to the needs of APHIS and can be classified into four broad efforts: 1) Detection and Delimitation, 2) Exclusion, 3) Control and Eradication and 4) Fundamental Biology.

I. Detection and Delimitation

Detecting, identifying and delimiting invading or expanding populations of fruit flies is the sine qua non of both the exclusion effort and the control and eradication efforts.

A. Attractants: All of the fruit fly eradication programs are based on our ability to detect the adult flies through the use of attractants. These attractants are classified into three major categories, 1) sex pheromones, 2) parapheromones, and 3) food- and host-derived attractants. The need for powerful attractants are acute if we are to prevent the establishment of any of these species. Research is devoted toward discovering new attractants or improving their usefulness.

1. Sex Pheromones: Most species of pest Tephritidae are thought to produce sex pheromones that may be useful as female attractants. However, success in developing pheromone-based lures has been limited. Attraction and orientation responses to fruit fly pheromones are poorly understood and the functions of numerous chemicals isolated and/or identified for some species are not well delimited.

The sex pheromone of the papaya fruit fly has been identified and a lure has been developed and tested for incorporation in a control scheme. Research is ongoing to isolate and identify attractive pheromones of several species, including the Mediterranean fruit fly, the Mexican fruit fly and the Caribbean fruit fly.

2. Parapheromones: "Parapheromone" is the name given to a group of compounds whose biological role in the life history of the flies is unclear. Thus far, the attractiveness of parapheromones to fruit flies are restricted to species of the genera Ceratitis and Bactrocera. No parapheromones have been confirmed for the Anastrepha genus.

Each of the known parapheromones, cuelure, Ceralure, trimedlure, and methyleugenol attracts a suite of related species with essentially no overlap--that is, they are specific attractants. Further, they are primarily male attractants although several attract unmated females under certain conditions. Some of the male lures are completely synthetic and do not occur in nature while others have been isolated as naturally occurring plant constituents such as the recently discovered male lure for the Malaysian fruit

fly. Plants that have these attractants are not necessarily either food sources or breeding hosts. None of the known male lures are produced by the insects themselves.

Parapheromones are used in detection programs and in some cases in eradication programs. Research is continuing in an effort to discover or design additional improvements in the male lures. Parapheromones for the Central and South American Anastrepha group would greatly enhance detection programs for these species.

3. Food- and Host-Derived Attractants: At the turn of the century, food attractants were based on sugary baits analogous to house fly attractants. The discovery of proteinaceous baits greatly improved attractant efficiency. These proteinaceous food attractants are plant by-products of industrial fermentation or fructose extraction processes. The active ingredients in these complex mixtures of hundreds of compounds have yet to be satisfactorily delineated. Recently, however, a chemically defined four-component bait which imitates the action of the complex proteinaceous natural product baits has been developed for the melon fly. This may lead to knowledge on how to enhance the attraction of food baits.

Chemicals derived from host fruit and foliage present an obvious area in which to look for female attractants. There is some evidence that such volatiles combined with the pheromones may act in a synergistic manner to increase attractiveness. The potential benefits of this research are important and research on them is currently very active.

The only widely used attractant for Anastrepha fruit flies are the proteinaceous food attractants. These proteinaceous food baits have a relatively short range of attraction. The need for improved attractants for this group of fruit flies is acute. Some leads to a fruit-derived attractant for the Mexican fruit fly (Anastrepha ludens) have been made and are under investigation.

- B. Traps, Devices and Formulations: Attractants are useless for detection unless they are deployed in an effective trap. Approximately \$15 million dollars per year are spent in maintaining detection traps for fruit fly invasions in the conterminous U.S. Servicing of these 116,000 traps is a significant effort and small improvements can provide significant cost benefits both in terms of actual expenses and in money saved through earlier detection of invading populations. Traps are evaluated in terms of convenience, cost, and efficiency. There is always a demand for better traps.

The use of multicomponent mixtures in pheromone baited traps will demand a balanced release rate for the components. Cost savings can also be effected through controlled release systems for parapheromone traps.

In addition to addressing the mechanics of trap and lure dispenser designs, research is also directed at evaluating the efficiency of trapping arrays. The probability of detecting the first invader will always be an imponderable, but experimental and statistical procedures can provide probability statements for detecting populations of a certain size. Research of this type could be incorporated into more science-based risk evaluations. To err and miss the discovery of the early generations of an infestation is costly but detection trap arrays are costly as well. An acceptable balance must be achieved.

- C. Host Fruit Surveys: A significant amount of commercial fruit enters the continental U.S. from fruit fly quarantined areas. This fruit is either grown in fly-free zones or is subjected to some sort of post-harvest treatment. A portion of each regulated shipment is subjected to physical examination to determine compliance and to detect any failures in the system. An approach under development is the use of highly sensitive microphones to listen to larval feeding. The efficiency of finding early instar larvae far surpasses the efficiency of fruit cutting. Development of an automated system for surveying large quantities of fruit is under consideration at this time. In any case, the improvement of the statistical probability of detecting infested fruit by simple fruit cutting must be refined.

- D. Systematics: Systematic data are basic to other biological studies and quarantine and control programs, all of which depend upon correct knowledge of what species are involved. Many gaps remain in our systematic knowledge of the Tephritidae, particularly in tropical regions where the majority of the major pests originate. More than 150 species of fruit flies have been identified as pests, of which about two dozen are considered to have major economic importance. These belong to genera that together include more than one thousand species, with many more yet to be discovered and described. Recognition of the pests from the other species, most of which are poorly known biologically, is thus a problem. Also, most taxonomy is based upon adult specimens and the immature stages are poorly known and difficult to identify. Finally, more thorough morphological studies and the use of new biochemical techniques have revealed that some of what were once thought to be widespread, polyphagous pest species (e.g. Oriental fruit fly; Anastrepha fraterculus) are actually complexes of very similar "cryptic" species. This has grave consequences for research on commodity treatments. The answers can come only through extended research involving classical morphological taxonomy and molecular techniques such as isozyme analysis or DNA

fingerprinting. Extensive field work to collect and rear specimens for study is also critical.

These molecular techniques may also help identify the point of origin of populations that invade the continental U. S. For example, did the medfly populations which invaded California in 1989-90 come from Hawaii, Central America, South America, Europe or elsewhere? Development of a catalog of DNA fingerprints or isozyme analyses of localized populations throughout the world may enable us to identify the geographical origin of invaders and to define the pathways of entrance and thus increase or modify the exclusion effort selectively.

ARS currently devotes 2 SY's toward molecular identification techniques and 1 SY toward classical systematics of fruit flies. These areas have a high priority and are understaffed in relation to their importance and needs. To cope with our fruit-fly problems, we must know the species with which we are dealing.

- E. Semiochemical Structure-Activity Relationships: The basis of all olfaction is an interaction between a chemical molecule and the surface of the receptor organelle of the insect and subsequent processing of the signal in the peripheral and central nervous system. A correlation exists between the geometry of the molecular structure and its activity as an attractant. If this structure-activity relationship is discerned, better attractants could be designed and synthesized. Perhaps the largest study of structure-activity relationships in tephritid fruit flies has been conducted on the isomers of trimedlure and its analogs. Trimedlure is the parapheromone that attracts the medfly. This work has led to the development of a more persistent attractant but has failed to produce a predictive hypothesis. However, the fact that an organism can detect a compound doesn't mean that the compound is an attractant unless the organism responds positively to it. This type of research requires a team of behaviorists, chemists and neurophysiologists.
- F. Basic Biology and Behavior: The natural basis for fruit fly attraction to lures used in detection programs is likely the orientation by the flies to food sources, host plants, and other flies as potential mates or competitors. The successful pursuit of natural attractants as lures and the discovery of new attractants depends to a great extent on our understanding of fruit fly orientation behavior and the significance of such behavior in the survival and reproduction of the fly. The pursuit of such knowledge, including a better understanding of how and why fruit flies respond to both natural and man-made attractants, is important to future successes in developing new detection technology.

II. Exclusion

- A. Postharvest Disinfestation: Postharvest quarantine treatment to disinfest fruit fly eggs or larvae from host fruit from infested areas is an important adjunct to exclusion inspection. Agricultural industries are dependent upon post harvest quarantine treatments using heat, cold or chemical fumigants to maintain fruit exports from infested areas.

The registration for the postharvest use of ethylene dibromide, one of the two most important chemical fumigants including methyl bromide, was withdrawn in 1984 because of possible detrimental effect to human health. Since then, physical treatments, such as hot water immersion or forced hot air, were developed for some fruits of export importance. Work with these treatment technologies continue for a number of different fruits and fruit fly species. However, latitude of such work is hampered by the universal principle whereby the time/dose relationship must be great enough to kill the insect but not so great as to injure the commodity. Conversely, some fruits such as carambola, grapefruit and litchi can be treated with cold temperatures for 10 to 20 days to eliminate fruit fly eggs and larvae. However, many fruits cannot tolerate the time/low temperature combinations required to achieve quarantine security.

The time/temperature combinations required to achieve quarantine security is known for some fruit fly species, whereas they are being studied for other species. These combinations must then be tested against the host fruits in both physiological and quality studies to determine fruit tolerance to the treatments. Other investigations include ways to enhance fruit tolerance to treatment, to lower treatment costs, and to reduce postharvest decay and loss of shelf life that result from treatment,

Irradiation is a viable alternative quarantine treatment for some host fruits such as litchi, papaya and mango, and the amount of radiation required to achieve quarantine security is documented. However, consumer acceptance remains the main obstacle to the successful use of irradiation as a quarantine treatment. Replacement of isotope source irradiator technology (cobalt or cesium) with electrical source (linear accelerator) technology may lead to consumer acceptance. Therefore, research with electrical source treatment facilities and consumer education is needed. Additionally, more research is needed on the electromagnetic spectrum to update previous work with microwaves, and research should be initiated into other areas of high energy physics to find new potential quarantine treatment methods.

- B. Fly-Free Zones and a Systems Approach to Quarantine Security: One approach to safeguarding commodities grown in regulated infested areas is the creation of "fly-free zones." Through the use of

survey traps, sanitation, wild-host destruction and other regulatory measures, fruits grown in certain areas can be approved for export commerce without treatment under certain compliance agreements. These programs have clearly written protocols to ensure that the risk of infestation is minimal. The current programs operate in areas that are biologically marginal for the respective fruit fly species.

Large-area population suppression programs would utilize the same techniques that have been developed for eradication programs but they would be used on a continuing sustained basis. The releases of sterile Mexican fruit flies in the lower Rio Grande valley of Texas is an example of such a program. Other programs may utilize various combinations of techniques such as male annihilation (MA) through mass trapping and sterile insects (SIT). Augmentative parasitoid releases combined with SIT or MA is another approach.

- C. **Total risk evaluation:** The concept of total risk evaluation needs to be developed. Quarantine level security in commercial agriculture in permanently infested areas can be achieved through a combination of techniques involving regulation of the variety and manner in which the crops are grown, fly population suppression, post-harvest treatments and an understanding of the distribution of low-level infestations in harvested fruit. This holistic or pest management approach to quarantine security may have wide application in international agriculture.

An important aspect of such a holistic approach is a thorough knowledge of the suitability of host species and varieties as fly breeding hosts. Varieties of susceptible host species vary widely in their suitability as fruit fly hosts. The biochemical and/or physical nature of this host plant resistance needs to be determined and enhanced through classical plant breeding and/or bioengineering or through biorational field treatments to enhance physiological resistance.

Thus, "exclusion" is still a fertile field of research with new ideas and approaches under development. ARS is devoting approximately 14 SY's directly toward these ends but the spin-off benefits from the other two broad thrusts, "Detection and Delimitation," and "Control and Eradication" are very high.

- D. **Inspection:** Tephritid fruit flies have been spread through the transportation of infested fruits and vegetables. Legal barriers in the form of agricultural quarantines have been established to exclude host-plant material originating in areas where these pests are known to occur. Inspection protocols have been established to enforce these quarantines. X-ray machines have been developed to detect fruit hidden in baggage and packages, but recent research has shown that they are far from foolproof. Research is needed to improve instrumental detection-based techniques. Chemical vapor

sampling for detecting aroma signatures of smuggled plant material is also a subject for research.

In addition, social research on the propensity of various sectors of the public to violate the legal quarantines could aid in the development of public awareness campaigns. Accordingly, research directed at developing ELISA techniques or other such markers would greatly aid in identifying the origin of infested fruits and thereby identify the most important avenues of invasion.

III. Control and Eradication

The goals of the ARS fruit fly research program are to provide the means to prevent the further spread of these pests; to provide the means for sustainable commercial agriculture in areas already infested ; and, ultimately, to provide acceptable means to eradicate incipient or established populations of fruit flies. There are three proven technologies for eradication. They are in decreasing order of pesticide usage: 1) proteinaceous food bait sprays utilizing ultra-low volume amounts of insecticide, 2) male annihilation technology using a parapheromone with an insecticide at between 1 or 2 orders of magnitude less than that used in bait sprays, and 3) sterile insect releases. These three techniques have been used alone or in combination to eradicate one or more species of fruit flies on a number of different occasions. Other technologies that are under development for control or eradication are female annihilation and augmentative parasitoid releases.

- A. Pesticides: Malathion has been used in proteinaceous food bait sprays as the toxicant of choice because of its relatively low mammalian toxicity. However, the search for safer, usable substitutes continues on other synthetic pesticides such as pyrethroids, microbial-derived toxicants, hormonal toxicants and inorganic boron.

Naled, an organophosphate insecticide, is used in male annihilation programs and in certain of the detection traps because of its rapid kill. Malathion can be substituted for it in male annihilation programs in most cases. Other toxicants are also under consideration for this use.

Soil drenches of diazinon are used to kill pupae in the soil under known infested trees in eradication programs directed against incipient invasions. Registration of diazinon for this use is being lost and other soil insecticides are being evaluated for incorporation as a component in eradication programs. In addition, research has been conducted on the use of insect attacking nematodes in aqueous suspensions as soil drenches for control of fruit fly larvae as they enter the soil to pupate.

- B. Male/Female Annihilation: The male annihilation technique is a proven technology for the Oriental fruit fly. Methyleugenol is the male lure for the Oriental fruit fly and a number of related species, such as the peach fruit fly. It is used as a food flavoring and appears on the "Generally Regarded as Safe" list of FDA. One study attributed carcinogenic properties to methyleugenol in a nonstandard test protocol. Consequently, a search was made to find substitute attractants. Out of hundreds of compounds screened, none were found to be as good as methyleugenol, but about five had an acceptable attraction. These compounds need to be evaluated in male annihilation use patterns as do the newly discovered male/female lures for other species.

The use of the sex pheromone of the papaya fruit fly in a female annihilation program is a novel technique that requires evaluation on a large scale. The use of sticky traps incorporating the male lures in the sticky formulation without an insecticide is being evaluated in a cooperative ARS-APHIS program against the medfly and may have use in other mass-trapping programs as a non-insecticidal technology.

- C. Sterile Insect Release Technology (SIT): This is the most environmentally acceptable technique, but it is also the most complex of the available eradication technologies, demanding the greatest resources and skill in its application. Its usage is strictly limited by fly production capacity. Although large numbers of flies are routinely reared, the exact nutritional needs of larvae have not been defined. Production crashes are an unfortunate fact of life in rearing operations. Sometimes the causes are easy to identify and at other times they are obscure. Research is being conducted to define the nutritional requirements, to regulate the microflora of the larval diet, and to mechanize handling operations. Waste disposal is a significant problem and expense in large-scale operations. Recycling or disposal procedures for the large volumes of spent larval diet are being investigated. Rearing procedures for other pest species, especially a number of Anastrepha species from Central and South America are being investigated. SIT is used routinely on the Mexican fruit fly and is being evaluated for the Caribbean fruit fly.

Behavioral quality of mass reared fruit flies is an important aspect of SIT. A system of standardized laboratory tests for medfly and Caribbean fruit flies have been available for years. These tests have a field equivalent where by they are correlated with similar tests in field cages and in the field. Ability to measure certain aspects of field effectiveness such as sperm competition, mating interactions in the field etc. lags behind. New sperm identification techniques offer promise for field performance evaluation. Other techniques to measure host and mate finding efficiency and mating competitiveness are needed.

Further improvements are required for sterilization procedures utilizing biophysical techniques other than gamma rays from isotope sources, improved marking procedures, controlled atmosphere packaging systems, and other aspects of the production system.

Other researchable problems are fly release systems with aircraft or ground equipment including aerial release of unchilled adults, reduction of sterilization dose to achieve greater competitiveness; single-sex releases; and, most importantly, data management and monitoring technology. For example, there is a debate about the effect of the simultaneous release of both sterile males and sterile females. Research is being conducted now with the medfly to determine whether the sterile females are beneficial, detrimental or neutral to the success of the program. This evaluation is only possible because mutational selection has lead to a strain in which the pupal color of the males and females differ and they can be sorted automatically.

More genetic research is needed to produce a strain with a sex-linked lethal factor that would enable selection to take place in the egg or early larval stage to save on rearing costs. If any nondestructive sorting procedure could be developed for use in the early larval stages, one sex could be used in the SIT releases and the other sex used to rear parasitoids as is being done in the Caribbean fruit fly/parasite program in Florida.

D. Biocontrol:

1. Pathogens: Pathogens of Diptera are few. Some work is being conducted on the exo- and endotoxins of Bacillus thuringiensis. Certain microbial diseases are known to occur in a fruit fly mass-rearing operation in Mexico, but no attempt has been made to see if they could be used as a mortality factor in bait sprays or other contexts. Studies have not been conducted on the effect of viruses up to now. Most diseases do not seem to develop into epidemic proportions, however. Larvae are attacked by nematodes but only during the brief period after they leave the fruit and before they pupate in the soil. Adults are able to avoid ingesting nematodes during feeding but are susceptible to invasion through sutures in the exoskeleton.
2. Parasitoids: About a dozen parasitoids of Old World tephritids have been established in Hawaii and Florida. Three or four of these introduced parasites have been found to have an impact on fruit fly populations and additional research is warranted. No substantial parasite explorations by ARS have been done for more than 30 years. The parasitoid complex of Anastrepha is not well known.

A few parasitoid species have been reared in large numbers on fruit fly hosts for use in augmentative and inundative releases. Combining parasitoid releases with sterile medflies and Caribbean fruit flies has shown promise as a control technique. The species chosen for this work are those that are easy to rear. Other species may be more effective for use of this technology but first improved rearing techniques must be developed. Augmentative parasitoid releases are conceptually new for fruit flies and should be pursued. This is a field for investigation with other species of fruit flies and parasitoids.

The biology of the parasitoids, their mating behavior and host-finding systems, are mostly unknown. Semiochemicals undoubtedly play a significant role in their biology and may be an avenue to enhance their effectiveness. At present, there is no way of assessing the adult population of parasites present in a locale.

IV. Fundamental Biology

- A. Ecology and Behavior: Studies on the ecology and behavior of fruit flies are limited. The concept of leks as part of the mating strategy of several important species was only discovered in 1978. The ramifications of this strategy on bioassays of pheromones and the sterile release programs are just now being examined. The diurnal rhythms of feeding, mating, and egg laying are known mostly from cage studies. The influence of light on behavior, especially mating, in field populations is mostly unexplored.

It has not been established whether an active dispersal phase occurs in the life history of any species. If there is such a phenomenon, it could greatly affect SIT strategies.

The spatial distribution of fruit flies on both a large and small scale resemble a Poisson distribution. The development of stratified sampling systems to determine population estimates and to guide SIT release patterns is needed. SIT eradication is dependent upon achieving suitable overflooding ratios throughout the area. Because concentrations of wild flies in hotspots must be overflooded, it is necessary to release very large numbers of sterile flies over the whole area. Better spatial and numerical estimates of the wild fly distribution is needed that could reduce costs of distributing the sterile flies.

- B. Physiology, Biochemistry and Genetics: Relatively little effort has been devoted to these fields until recent times. SIT programs are guided by generational day-degree models that are based on forty-year old data. A reevaluation of the day-degree

developmental requirements for some of these species is being conducted but for others, no data are available.

The hormonal and endocrine systems of fruit flies have not been studied to any extent. A researchable area involves the determination of the role of neuropeptides in the accessory glands in extending the refractory period after mating. If they, indeed, control the refractory period, it would be important to monitor the levels of these neuropeptides in males in the mass rearing colony to assure that long refractory periods occur after they mate with wild females.

Relatively little histological research has been done. Nutritional research is largely with semi-defined diets and the role of symbiotic microflora has never been satisfactorily clarified.

The electrophysiological studies of the antennal olfactory receptors should provide some of the answers to semiochemical problems. Antennograms have provided interesting data but much more work at the level of single receptor neurons is needed to increase our understanding of structure- activity relationships in semiochemical responses.

Genetic analyses and manipulation offers a great potential towards understanding the general biology of fruit flies as well as, more specifically, monitoring and controlling populations. This is reflected in current programs using DNA analyses to distinguish species and strains, and in more prospective uses in genetic sexing, sterilization and marking. Continued progress requires concerted efforts in fundamental genetics research, including both classical and molecular mechanisms.

- C. Environmental and Health Relationships: The chief concern of large eradication projects is the effect that they may have on non-target insects and on the environmental fate of pesticides and/or semiochemicals. The initial work on any new semiochemical in terms of development of analytical techniques is conducted by ARS but, for the most part, the approval to use them in the field is usually required by outside agencies.
- D. Non-technical Issues: Because the ultimate objectives of these programs are to develop eradication systems, research necessarily involves large-area applications at the final stages of development. Some of these Such programs, depending upon their size and location, infringe to a greater or lesser extent on the public. Considerable resources have been dedicated to such programs by APHIS. An example has been the Africanized Bee program. In addition, APHIS has increased the amount of human resources available to these projects since 1988. The legal issues for compliance with environmental laws and regulations are becoming increasingly complex. The size of pilot-scale programs

necessarily involve administrative, logistical, and personnel issues of increasing complexity. There are important unresolved policy issues in regard to the registration of novel compounds or uses in those programs. Thus, along with the increase in the complexity of the science issues of fruit fly eradication has come an increase in administrative issues as well as a much greater need for public support.

USDA-ARS ACTION PLAN FOR FRUIT FLY RESEARCH

I. Detection and Delimitation

A. Pheromones

Background - The only pheromone that has been identified, synthesized and successfully field tested is the pheromone of the papaya fruit fly. There has been considerable work on identifying and synthesizing the pheromone components of the Mediterranean fruit fly, the Caribbean fruit fly, the Mexican fruit fly and the West Indian fruit fly, but optimum blends of pheromone components have not been formulated or field tested.

Needs - Fruit fly pheromones offer considerable promise as selective and potent attractants for female or male flies. An effective gender- and species-specific attractant for the female medfly would be of considerable value in evaluations of ongoing mass releases of sterile flies. At present, no practical attractant for female medflies is available.

1. Mediterranean fruit fly

I.A.1 Landolt, P. J.

CRIS 6615-22000-009-00D

SY: 0.2

Objective: Development of a pheromone-based lure for the medfly.

Significance: The male medfly pheromone is known to be attractive to females, which are relatively unresponsive to other medfly lures. Such technology would provide an additional detection tool, and may also be useful in developing new control or eradication technology based on female annihilation.

Justification: Industry - I.B, III.A; Regulatory - I.A, I.D.

Constraints: Methods are needed to formulate required pheromone chemicals for additional studies using pheromone as a trap lure.

References: None.

Co-investigators/Cooperators: R. Heath, ARS, Gainesville; APHIS S&T, Guatemala City.

Work Plans for FY 92: Reassessment of active male pheromone chemicals for attractiveness to females and

determination of optimum synthetic blend for attractiveness.

I.A.1 Flath, R. A.

CRIS 5325-22000-010-00D

SY: 0.1

Objective: Semiquantification and examination of male medfly calling emissions.

Significance: In their efforts to assess the relative importance of male medfly calling emission components in females, cooperators need improved quantitative data. Variations in compositional makeup with other variables such as time of day, insect age, etc., may also be significant.

Justification: Industry - I.B; Regulatory - I.A, I.D.

Constraints: None significant, other than inherent limitations of available collection and analytical techniques.

References:

Jang, Light, Flath, et al. 1989. EAG responses of the Mediterranean fruit fly, *Ceratitis capitata* to identified volatile constituents for calling males. Entomol. Exp. Appl. 50:7-19.

Co-investigators/Cooperators: D. M. Light, ARS, Albany; E. B. Jang, ARS, Hilo. Potential: R. R. Heath, ARS, Gainesville.

Work Plans for FY 92: Headspace comparison of male emissions from irradiated vs. fertile Hawaiian lab-reared flies.

I.A.1 Jang, E. B.

CRIS 5320-22000-005-00D

Liquido, N. J.

Also: I.A.3

SY: 0.2 (Jang), 0.1 (Liquido)

Objective: Development of effective pheromone-based female attractants for Mediterranean fruit fly, oriental fruit fly, melon fly and Malaysian fruit fly. Assess the chemoreceptive basis of how pheromonal compounds promote, drive and modulate behavior of female fruit flies.

Significance: Male attractants have been found for all above species and along with protein baits show some

attractancy to females. However, development of a pheromonal attractant for females would greatly augment current survey and detection protocols especially in areas of new introductions, and could be developed into a species specific method for detection and eradication technology.

Justification: Industry - I.B, II.C, III.H; Regulatory - I.A, I.D.

Constraints: None.

References:

- Jang, E. B., D. M. Light, R. A. Flath, J. T. Nagata and T. R. Mon. 1989. Electroantennogram responses of the Mediterranean fruit fly, Ceratitis capitata to identify volatile constituents from calling males. Entomol. Exp. Appl. 50:7-19.
- Jang, E. B. and K. A. Nishijima. 1990. Identification and attractancy of bacteria associated with Dacus dorsalis (Diptera:Tephritidae). Environ. Entomol. 19:1726-1731.
- Jang, E. B. 1990. Effects of mating on olfactory behavior of female medfly to male pheromone or host fruit odors. In: Proc. Intl.Sympos. Fruit Flies of Econ. Importance. Antigua, Guat.
- Dickens, J. C., E. B. Jang, D. M. Light and A. R. Alford. 1990. Green leaf volatiles enhances insect pheromone response. Naturwissenschaften 77:29-31.

Co-investigators/Cooperators: D. M. Light, R. A. Flath, R. Teranishi, ARS, Albany. Potential: B. Leonhardt, ARS, Beltsville; APHIS S&T; CDFA.

Work Plans for FY 92: I.D. pheromonal components, blends and ratios attractive to females. Assess effects of physiology on response to pheromone.

I.A.1 Heath, R. R.

CRIS 6615-22000-008-07R

SY: 0.3

Objective: Optimization of a pheromone trapping system for the Mediterranean fruit fly.

Significance: Optimization of this system may lead to an effective system for removal of female flies. This may provide an alternative to the use of halogenated parapheromones which are ineffective at capturing female flies. A female attractant may provide an opportunity for developing female annihilation systems.

Justification: Industry - I.B, III.C, III.G;
Regulatory - I.A, I.B, I.D, I.H.

Constraints: None.

References:

Heath, R. R., P. J. Landolt, J. H. Tumlinson, D. L. Chambers, R. Murphy, R. E. Doolittle, B. D. Dueben, J. Sivinski and C. O. Calkins. Analysis, synthesis, and formulation of sex pheromone components of the male Mediterranean fruit flies attractive to females. J. Chem. Ecol. (In press).

Co-investigators/Cooperators: P. J. Landolt and N. D. Epsky, ARS, Gainesville; APHIS S&T, Guatemala; E. Jang, ARS, Hilo; T. Millar and T. Baker, Riverside; R. A. Flath, ARS, Albany.

Work Plans for FY 92: Isolate and identify additional pheromone components that will increase the attractiveness of the three component blend. Delineate the importance of the individual components of the three component blend. Investigate and improve methods for formulation of the three component blend.

I.A.1 Light, D.

CRIS 5325-22000-010-00D

Also: I.A.3

SY: 0.2

Objective: Identification and development of effective pheromonal attractants for unmated female tephritids endemic to Hawaii, the Mediterranean fruit fly, oriental fruit fly, melon fly and Malaysian fruit fly. Assess the chemoreceptive bases of how these odors promote, drive and modulate lek and mate selection, courtship, and mating behaviors of female fruit flies.

Significance: Gender-specific attractants have been developed for males of many tephritid species but are lacking at present for females. Development of a species-specific (pheromonal) female attractant would greatly augment current survey and detection protocols especially in areas of new introductions and could be developed into a species and gender-specific method for detection and eradication technology.

Justification: Industry - I.B, III.G, III.H;
Regulatory - I.D, I.E, I.F, III.N, III.O, III.V.

Constraints: None.

References:

Jang, E. B., D. M. Light, R. A. Flath, J. T. Nagata and T. R. Mon. 1989. Electroantennogram responses of the Mediterranean fruit fly, Ceratitidis capitata, to identified volatile constituents from calling males. Entomol. Exp. Appl. 50:7-19.

Dickens, J. C., E. B. Jang, D. M. Light and A. R. Alford. 1990. Enhancement of insect pheromone responses by green leaf volatiles. *Naturwissenschaften* 77:29-31.

Co-investigators/Cooperators: E. B. Jang, ARS, Hilo; R. A. Flath, R. Binder, ARS, Albany. Potential: N. J. Liquido, ARS, Hilo.

Work Plans for FY 92: Trap the headspace pheromonal emissions of wild, lab-fertile and lab-sterile male medflies (See Flath I.A.1 for identification and analysis); continue to determine and assess the behavioral activity and synergism of major, intermediate and minor components of the pheromonal male medfly emission; continue studies on host plant volatiles enhancing the responses to medfly pheromone; and continue trapping and analyses of pheromonal emissions of both oriental and melon fruit flies.

2. Anastrepha

a. A. ludens

I.A.2.a Heath, R. R. *CRIS 6615-22000-008-07R*

SY: 0.2

Objective: Develop a pheromone trapping system for Anastrepha ludens.

Significance: The development of a pheromone based female trapping system will result in an efficacious method for monitoring female flies. Current methods using proteinaceous baits are not effective. A female attractant may provide an opportunity for development of a female annihilation system.

Justification: Industry - I.A, III.C, III.G;
Regulatory - I.A, I.B, I.D.

Constraints: Chemicals will have to be synthesized and a formulation methods for these chemicals will have to be developed.

References: None.

Co-investigators/Cooperators: D. Robacker and R. Mangan, ARS, Weslaco; N. D. Epsky, ARS, Gainesville; APHIS S&T, Guatemala.

Work Plans for FY 92: Isolate and identify the pheromone components released by sexually mature male fruit flies

during the photophase. Work with entomologists to facilitate the development of a bioassay to determine the attractiveness of the pheromone blend to female Mexican fruit flies.

I.A.2.a Landolt, P. J. CRIS 6615-22000-009-00D

Also: I.A.2.b

SY: 0.05

Objective: Development of sex pheromone bioassays lures for Anastrepha species.

Significance: Trapping methods available for pest species of Anastrepha are generally considered weak. Development of new lures for these flies could greatly improve the ability of agencies to detect introductions and infestations and to monitor populations in fly-free zones.

Justification: Regulatory - I.A, I.D.

Constraints: Suitable attraction bioassays are not developed or available for evaluating pheromone blends and lures for any species of Anastrepha. Basic behavioral studies of fly responses to signaling males are necessary to determine what attraction responses occur and how to test for them in the lab. Chemicals identified from these species that may be pheromones are not available for tests. Methods of chemical synthesis and lure formulations are needed for a number of these compounds.

References: None.

Co-investigators/Cooperators: R. Heath and J. Sivinski, ARS, Gainesville; APHIS S&T, Guatemala; D. Robacker, ARS, Weslaco.

Work Plans for FY 92: Develop a flight tunnel bioassay for studying orientation behavior and for evaluating sex pheromone blends for attractiveness to females. Also, see Heath, R. R. for pheromone isolation and identification.

I.A.2.a Robacker, D. CRIS 6204-43000-005-00D

SY: 0.2

Objective: Determine biological functions of two new putative pheromone components produced by calling male

Mexican fruit flies recently identified by R. Heath (ARS) and M. Battiste (Univ. of Florida, Gainesville, FL).

Significance: The information will be used to determine which of the seven chemicals produced by male flies are necessary to develop a pheromone-based trapping system.

Justification: Industry - I.A; Regulatory - I.A, I.D, III.D.

Constraints: Determination of isomerism of suspensolide and synthesis of macrocyclic lactones.

References:

Robacker, D. C. 1988. Behavioral responses of female Mexican fruit flies, Anastrepha ludens, to components of male-produced sex pheromone. J. Chem. Ecol. 14:1715-1726.

Co-investigators/Cooperators: R. R. Heath, ARS, Gainesville. Potential: P. J. Landolt, ARS, Gainesville; A. DeMilo, ARS, Beltsville.

Work Plans for FY 92: All of above.

b. Other Anastrepha Species

I.A.2.b Sivinski, J. CRIS 6615-22000-009-00D

Also: I.C.1.a; I.C.2.a; I.D.2

SY: 0.1

Objective: Development of traps and attractants for the Caribbean fruit fly.

Significance: Present traps and attractants for Anastrepha spp. are relatively ineffective. Pheromones and host attractants when combined with suitable traps will provide a better means of monitoring populations or through the capture of females, an alternative means of control.

Justification: Industry - I.A; Regulatory - I.A, I.D.

Constraints: Funding may prove to be a difficulty, particularly if host-plant volatiles require substantial analysis.

References:

Webb, J. C., T. Burk and J. Sivinski. 1983. Attraction of female Caribbean fruit flies, Anastrepha suspensa (Diptera: Tephritidae), to the presence

- of males and male-produced stimuli in field cages. *Ann. Entomol. Soc. Am.* 76(6):996-998.
- Sivinski, J. M. and R. R. Heath. 1988. Effects of oviposition on longevity, remating and pheromone response in the Caribbean fruit fly Anastrepha suspensa (Loew). *Ann. Entomol. Soc. Amer.* 8:2021-2024.
- Sivinski, J. 1989. Color spherical traps for the capture of the Caribbean fruit fly Anastrepha suspensa (Loew). *Fla. Entomol.* 73:123-128.
- Sivinski, J. M. and C. O. Calkins. 1986. Pheromones and parapheromones in the control of tephritids. *Fla. Entomol. Symp. Series.* 69:157-168.

Co-investigators/Cooperators: R. Baranowski, Univ. of FL, Gainesville; R. Heath, N. Epsky and P. J. Landolt, ARS, Gainesville.

Work Plans for FY 92: Field tests of various traps exposed to flies of various sexes, strains and conditions will continue. Host volatiles will be collected.

I.A.2.b Heath, R. R. CRIS 6615-22000-008-07R

SY: 0.1

Objective: Develop a pheromone trapping system for Anastrepha suspensa.

Significance: The development of a pheromone-based female trapping system will result in an efficacious method for monitoring female flies. Current methods using proteinaceous baits are not effective. A female attractant may provide an opportunity for development of a female annihilation system.

Justification: Industry - I.A, III.C, III.G;
Regulatory - I.A, I.B, I.D.

Constraints: Additional chemical analyses of the pheromone of these flies is needed. Some chemicals will have to be synthesized and a formulation method for these chemicals will have to be developed. Bioassays will be needed to determine the efficacy of the pheromone blend. Field tests will be needed.

References:

- Chuman, T., J. M. Sivinski, R. R. Heath, C. O. Calkins and J. H. Tumlinson. 1988. Suspensolide, a male-produced sex pheromone component of Caribbean fruit fly (Anastrepha suspensa [Loew]). *Tetrahedron Lett.* 29(50):6561-6564.

Co-investigators/Cooperators: C. O. Calkins, P. J. Landolt, J. Sivinski and N. D. Epsky, ARS, Gainesville.

Work Plans for FY 92: Determine when the reported putative pheromonal components are released by sexually mature males during the photophase. Work with

entomologists to facilitate the development of a bioassay to determine the attractiveness of the pheromone blend to female Mexican fruit flies. Determine the effect on food source on pheromone release by male flies.

Also see Landolt I.A.2.a.

3. Bactrocera (Dacus)

I.A.3 Leonhardt, B. A. CRIS 1275-22000-089-00D

SY: 0.1

Objective: Isolate, identify and synthesize the pheromone of Dacus latifrons, the Malaysian fruit fly.

Significance: The Malaysian fruit fly is established in Hawaii and could be introduced into the continental U. S. A synthetic attractant (latilure) has been identified for males but the addition of the pheromone to the trap bait would allow detection of female flies as well.

Justification: Regulatory - I.A.

Constraints: None.

References: None.

Co-investigators/Cooperators: E. Jang and R. T. Cunningham, ARS, Hilo. Potential: California Dept. of Food and Agriculture; ARS.

Work Plans for FY 92: Establish bioassay; test collected fractions.

Also see Jang and Liquido I.A.1, Light I.A.1.

4. Toxotrypana

References:

- Landolt, P. J., R. R. Heath, and J. R. King. 1985. Behavioral responses of female papaya fruit flies, Toxotrypana curvicauda Gerstaecker (Diptera: Tephritidae), to male-produced sex pheromones. Ann. Entomol. Soc. Am. 78: 751-755.
- Chuman, T., P. J. Landolt, R. R. Heath, and J. H. Tumlinson. 1987. Isolation, identification and synthesis of a male-produced sex pheromone of the papaya fruit fly, Toxotrypana curvicauda Gerstaecker (Diptera: Tephritidae). J. Chem. Ecol. 13(9):1979-1982.
- Landolt, P. J. and R. R. Heath. 1988. Effects of age, mating, and time of day on behavioral responses of female papaya fruit fly, Toxotrypana curvicauda Gerstaecker, to synthetic sex pheromone. Environ. Entomol. 17(1):47-51.
- Landolt, P. J., R. R. Heath, H. R. Agee, J. H. Tumlinson and C. O. Calkins. 1988. A sex pheromone-based trapping system for the papaya fruit fly,

- Toxotrypana curvicauda Gerstaecker (Diptera: Tephritidae). J. Econ. Entomol. 81 (4): 1163-1169
- Landolt, P.J., and R.R. Heath. 1990. Effects of Pheromone Release Rate and Time of Day on catches of Male and Female Papaya Fruit Flies (Diptera: Tephritidae) on Fruit Model Traps Baited with Pheromone. J. Econ. Entomol. 85(5):2040-2043.
- Landolt, P. J., R. R. Heath and H. R. Agee. A Novel System for Monitoring and Controlling the Papaya Fruit Fly. Patent # 4,992,268. 2/12/91. (Patent)

B. Parapheromones

Background - Medfly: Trimedlure is the accepted attractant for male medflies and is the standard for ranking the effectiveness of other fruit fly attractants. Ceralure is an iodinated-analog of trimedlure that exhibits the same attractiveness as trimedlure, but has greater persistence. Alpha-copaene and/or other naturally-occurring terpenoids show promise as male medfly attractants.

Oriental fruit fly: Methyleugenol, a naturally-occurring compound, is a very effective male attractant, and there is no great need for an alternative, unless the compound is clearly proven to be a carcinogen.

Melon fly: Cuelure is an effective and satisfactory attractant for the melon fly and no replacement is needed now.

Malaysian fruit fly: A group of related naturally-occurring compounds have recently been found to be attractive to the male Malaysian fruit fly. Latilure is the most effective of these compounds, but has not been field-tested extensively as yet.

Needs - Formulation of ceralure in solid dispensers needs to be explored for possible use in medfly traps. Additional work on alpha-copaene should be carried out to determine its potential for use in medfly detection. Perhaps an alternative for methyleugenol may be required. An attractant for Mexican fruit fly is needed. Solid dispensers are required for methyleugenol, cuelure and latilure. More effective attractants for all species are desired.

1. Mediterranean fruit fly

a. Trimedlure

I.B.1.a Warthen, Jr., J. D. CRIS 1275-22000-086

Also: I.G

SY: 0.1

Objective: To investigate the relative attractiveness of the eight trimedlure isomers versus the structural similarities of these eight isomers by computer molecular modeling.

Significance: The discovery of structure-activity relationships should aid in the synthesis of more potent medfly attractants.

Justification: Industry - I.B; Regulatory - I.D, I.M.

Constraints: None.

References:

- Warthen, et al. Molecular comparisons of trimedlure isomers by computer-aided molecular modeling. 25th MARM, ACS, #257, May 21-23, 1991, Newark, DL.
 McGovern, et al. Relative attraction of the Mediterranean fruit fly (Diptera: Tephritidae) to the eight isomers of trimedlure. J. Econ. Entomol. 83:1350-1354.
 Warthen and McGovern. 1988. Semi-preparative high performance liquid chromatographic separation of trimedlure isomers. Chromatographia 25:811-814.

Co-investigators/Cooperators: A. B. DeMilo and W. Schmidt, ARS, Beltsville.

Work Plans for FY 92: Establish model for covariance and complete manuscript for publication.

b. Ceralure

I.B.1.b DeMilo, A. B. CRIS 1275-22000-089

SY: 0.1

Objective: Develop methods to enhance the stability of the medfly attractant ceralure.

Significance: Ceralure's demonstrated exceptional residual attractancy makes it a prime candidate for use in the male annihilation technique. However, unlike trimedlure, ceralure decomposes during synthesis and in unprotected storage. If factors that influence ceralure's decomposition can be identified and controlled, then considerable cost savings will be realized for its commercial production as well as its prolonged field performance and shelf life.

Justification: Industry - I.B, III.B; Regulatory - I.D, III.F, III.H.

Constraints: None.

References: None.

Patent: McGovern, T. P. and R. C. Cunningham.
Persistent attractants for the Mediterranean fruit fly,
method of preparation and the method of use. U. S.
Patent 4,764,366. 8/16/88.

Co-investigators/Cooperators: R. T. Cunningham, ARS,
Hilo; J. D. Warthen, Jr. and B. A. Leonhardt, ARS,
Beltsville.

Work Plans for FY 92: Conduct aging studies with
ceralure neat, and in presence of photodecomposition
inhibitors. Determine influence of these stabilizers on
isomer distribution.

I.B.1.b Warthen, Jr., J. D. CRIS 1275-22000-086

Also: I.G

SY: 0.1

Objective: To investigate the relative attractiveness of
the four trans-ceralure isomers versus the structural
similarities of these four isomers by computer molecular
modeling.

Significance: Establishing structure-activity
relationships should aid in explaining why the B1
ceralure isomer is the most active iodo-isomer and the C-
trimedlure isomer is the most active chlorinated-isomer,
but yet have equal initial activity. This could aid in
the synthesis of more potent medfly attractants.

Justification: Industry - I.B, III.B; Regulatory - I.D,
I.M, III.F.

Constraints: None.

References:

Warthen and McGovern. Semi-preparative high-performance liquid chromatographic
separation of ceralure and related isomers. Chromatographia 29:135-138.

Co-investigators/Cooperators: A. B. DeMilo and W.
Schmidt, ARS, Beltsville.

Work Plans for FY 92: Manuscript to be completed by
DeMilo on relative field attractiveness.

c. α -Copaene and Related Sesquiterpenes

I.B.1.c Flath, R. A.

CRIS 5325-22000-010-00D

Also: I.B.1.d

SY: 0.65

Objective: Identification of more effective lures for the male Mediterranean fruit fly, based upon components of essential oils or commercial aroma formulations found to be attractive to the male.

Significance: Medfly detection and eradication programs would benefit from availability of more highly attractive lures. Such materials would likely have a greater effective range and would be more applicable to male annihilation programs.

Justification: Industry - I.B; Regulatory - I.A, I.D.

Constraints: If the attractive components of essential oil source materials are found to be sesquiterpenoid, their ultimate usefulness on a large scale will depend on the costs involved in concentrating the materials from their sources, or in synthesizing either the compounds themselves or active analogs of the compounds.

References: None.

Co-investigators/Cooperators: R. T. Cunningham, ARS, Hilo; A. B. DeMilo and J. D. Warthen, Jr., ARS, Beltsville.

Work Plans for FY 92: Fractionation and bioassay of screened essential oils, to isolate and identify attractive components.

I.B.1.c DeMilo, A. B.

CRIS 1275-22000-089

Also: I.G

SY: 0.1

Objective: Develop potent medfly attractants derived from alpha-copaene.

Significance: Alpha-Copaene, a plant-derived sesquiterpene, is a potent attractant for the medfly. As a male attractant, alpha-copaene ((+) enantiomer), appears to be more potent than trimedlure. The structure of alpha-copaene is complex and consequently it is unlikely that a commercial synthesis to provide large quantities will be economically feasible. Substructures

of alpha-copaene or structurally related compounds will be investigated as potential medfly attractants. Hopefully, less complex, easily synthesizable alternatives to alpha-copaene will be found. The discovery of such a compound(s) would substantially improve the detection and possibly control of this pest.

Justification: Industry - I.B, III.G; Regulatory - I.D, I.G.

Constraints: None.

References: None.

Co-investigators/Cooperators: R. T. Cunningham, ARS, Hilo; R. A. Flath, ARS, Albany; J. D. Warthen, Jr., ARS, Beltsville.

Work Plans for FY 92: Continue computer molecular modeling with α -copaene, trimedlure and ceralure. Compare molecular models with 3D structure of simple terpenes and sesquiterpenes. Review correlations and select one or two sesquiterpene models for synthetic probes.

d. Other

I.B.1.d DeMilo, A. B. CRIS 1275-22000-089

SY: 0.1

Objective: Develop, through synthesis and structure-activity studies, potent attractants for fruit flies (Mediterranean, oriental, melon, Malaysian).

Significance: New technologies being developed to control the medfly usually require unique physical and/or chemical properties of the attractant. Should any of the standard attractants in current practical or experimental use fail to meet regulatory or performance criteria, back-up attractants will be needed to insure continuity in detection and eradication programs.

Justification: Industry - III.B; Regulatory - I.D.

Constraints: None.

References:

McGovern, T. P. and R. T. Cunningham. 1988. Attraction of Mediterranean fruit flies to analogs of selected trimedlure isomers. J. Econ. Entomol. 81:1052-1056.

Patents: McGovern, T. P. and R. T. Cunningham.
Persistent attractants for the Mediterranean fruit fly,
the method of preparation and method of use. U. S.
Patent No. 4,761,280. 8/2/88.

McGovern, T. P., R. A. Flath, and R. C. Cunningham.
Attractants for Dacus latifrons, the Malaysian fruit fly.
U. S. Patent No. 4,877,607. 10/31/89.

Co-investigators/Cooperators: R. T. Cunningham and E.
Jang, ARS, Hilo; B. A. Leonhardt and J. D. Warthen, Jr.,
ARS, Beltsville.

Work Plans for FY 92: Continue structure-activity
relationship studies on oxa- and dioxaspiro compounds
(medfly attractants). Synthesize additional trimedlure
and ceralure analogs in response to new leads.

I.B.1.d Cunningham, R. T. CRIS 5320-22000-005-00D

SY: 0.1 (Cunningham), 0.1 (Warthen)

Objective: Field bioassays of an inherently more
attractive male lure for the medfly from analogs of
alpha-copaene and other sesquiterpenes.

Significance: Ceralure, although much more persistent
than trimedlure, does not produce a much wider sphere of
attraction. Alpha-copaene, a naturally occurring
tricyclic sesquiterpene, produces greater attraction but
it is too expensive and/or too difficult to synthesize to
be practical. If a relatively cheap analog can be
discovered, it would greatly enhance both detection and
eradication programs.

Justification: Industry - I.B, III.G; Regulatory - I.A,
I.D.

Constraints: None.

References: None.

Co-investigators/Cooperators: R. A. Flath, ARS, Albany,
A. B. DeMilo and J. D. Warthen, Jr., ARS, Beltsville.

Work Plans for FY 92: Field bioassay of alpha-copaene
and other sesquiterpenes.

Also see Flath I.B.1.c.

2. Oriental Fruit Fly

a. Methyleugenol**b. Alternates***I.B.2.b DeMilo, A. B.**CRIS 1275-22000-089*

Also: III.B.1.a.0.1

SY: 0.1

Objective: Develop potent attractants for the oriental fruit fly (B. dorsalis): alternatives to methyleugenol.

Significance: Methyleugenol (ME) is widely used as a standard lure to detect and control the oriental fruit fly. Abolishment of ME would severely hamper control of B. dorsalis and its absence would have enormous impact on the fruit industry in CA. Clearly, development of a safe attractant that could effectively replace ME could save agricultural communities threatened by the oriental fruit fly.

Justification: Industry - III.G; Regulatory - I.D.

Constraints: None.

References: Manuscript in progress.

Co-investigators/Cooperators: R. T. Cunningham, ARS, Hilo; B. A. Leonhardt and J. D. Warthen, Jr., ARS, Beltsville.

Work Plans for FY 92: Develop a method to synthesize a highly attractive ethyl analog of ME. Continue synthesis of analogs involving modification on the ring and ether moieties of ME.

*I.B.2.b Liquido, N. J.
Cunningham, R. T.*

CRIS 5320-22000-005-00D

SY: 0.1 (Liquido), 0.1 (Cunningham)

Objective: Investigation of methyleugenol analogs and other compounds to act as a substitute for methyleugenol in trap detection and male annihilation programs.

Significance: The use of methyleugenol might be prohibited because of its possible carcinogenic property. Methyleugenol is the basis of a system used in detecting and eradicating annual introductions of oriental fruit fly into mainland United States. The loss of the use of

methyleugenol would mean the inevitable establishment of oriental fruit fly in Southern California.

Justification: Industry - III.G; Regulatory - I.A, I.D, I.F, I.I., III.F.

Constraints: A considerable amount of work is needed in the field screening phase. Furthermore, a convincing pilot test on efficacy must be carried out in at least 1 km² experimental area. There is no commercial source of methyleugenol analogs. Tier I toxicological data are necessary for an EUP.

References: None.

Co-investigators/Cooperators: E. Jang, ARS, Hilo; A. B. DeMilo, J. D. Warthen, Jr., and B. A. Leonhardt, ARS, Beltsville. Potential: R. A. Flath and R. Teranishi, ARS, Albany.

Work Plans for FY 92: Initiate field bioassay of newly synthesized, possible methyl eugenol substitute.

I.B.2.b Warthen, J. D. CRIS 1275-22000-086

SY: 0.1

Objective: To investigate the separation of oriental fruit fly attractants 4 (and 5)-allyl-2-ethoxy-1-methoxybenzene and 4 (and 5)-allyl-2-ethoxy-1-phenol.

Significance: A substitute for methyleugenol is needed. Presently, the use of methyleugenol as an attractant prevents the establishment of the oriental fruit fly in Southern California.

Justification: Industry - I.C; Regulatory - I.D, I.F.

Constraints: None in initial screening phases.

References: None.

Co-investigators/Cooperators: A. B. Demilo and B. A. Leonhardt, ARS, Beltsville; R. T. Cunningham, ARS, Hilo.

Work Plans for FY 92: Utilize new HPLC columns for separation development.

3. Melon Fruit Fly

a. Cuelure

4. Malaysian Fruit Fly

a. Latilure

I.B.4.a Flath, R. A.

CRIS 5325-22000-010-00D

Also: I.C.2.c

SY: 0.05

Objective: Investigation of attractants for the Malaysian fruit fly.

Significance: Analogs of latilure, the present male lure for the Malaysian fruit fly, might prove useful in certain situations. This relationship needs investigation because of the potential threat of this fly to mainland agriculture. In addition, host plant-derived volatiles have potential as attractants for the female.

Justification: Regulatory - I.A, I.D.

Constraints: None.

References: None.

Patent: No. 4/877/607. McGovern, Cunningham, Flath. 1989. "Attractants for Dacus latifrons, the Malaysian fruit fly."

Co-investigators/Cooperators: R. T. Cunningham, ARS, Hilo; A. B. DeMilo, ARS, Beltsville.

Work Plans for FY 92: Some efforts on latilure analogs/derivatives as improved male lures. Insufficient time for work on host-derived female attractants this year.

b. Other

5. Anastrepha

a. A. ludens and obliqua

I.B.5.a Moreno, D. S.

CRIS 6204-43000-003-00D

SY: 0.3

Objective: Screen synthetic compounds as possible attractants for the Mexican fruit fly and West Indian fruit fly.

Significance: The viable attractants for the genus Anastrepha are proteinaceous food baits used in the McPhail trap. A better attractant for the Mexican fruit fly would make detection, monitoring and control of this fruit fly not only easier but more efficient. Currently thousands (probably more than 10,000) of traps are used almost continuously in California and Texas and in the norther states of Baja California Norte, Baja California Sur, Sinaloa, Sonora, Chihuahua, Nuevo Leon and Tamaulipas in Mexico. Many times, by the time the fruit fly is detected in traps, fruit infestation has already occurred. With a better attractant this problem should be greatly minimized.

Justification: Industry - I.A; Regulatory - I.A, I.D.

Constraints: None.

References: None.

Co-investigators/Cooperators: R. L. Mangan, ARS, Weslaco, Texas; A. B. DeMilo and B. A. Leonhardt, ARS, Beltsville, Maryland. Potential: APHIS S&T, Mission, Texas; SARH, CINIFAP, General Teran, Mexico.

Work Plans for FY 92: Modify a room to control temperature, humidity and lighting. Construct two-cubic-meter aluminum cages to contain flies for bioassays. Work out methodologies of handling insects for testing, including food substances, presentation of attractants to test flies and age structure of flies for tests. Standardize a bioassay system using Nu-Lure as the attracting standard. Measure best diel response of test flies. Determine maximum number of treatments that can be evaluated at the same time and in a single day. Initiate screening of synthetic compound.

I.B.5.a DeMilo, A. B.

CRIS 1275-22000-089

Also: III.B.1.e

SY: 0.1

Objective: Develop synthetic fruit fly attractants for the Mexican and West Indian fruit fly.

Significance: Current lures used to survey Anastrepha species are capable of trapping only a small percentage of existing populations. Since the 1960's, very few improvements have been made to increase efficiency of commercial lures. Consequently, there is a need to

develop more efficient attractants that can be employed in surveillance and/or eradication programs.

Justification: Industry - I.A, III.G; Regulatory - I.D, III.UU.

Constraints: None with regard to the chemical input to the initial phases of screening. However, quick, safe and reliable laboratory bioassay procedures must be developed to screen candidate attractants before this project can begin.

References: None.

Co-investigators/Cooperators: D. S. Moreno and R. L. Mangan, ARS, Weslaco; J. D. Warthen, Jr. and B. A. Leonhardt, ARS, Beltsville.

Work Plans for FY 92: Review earlier bioassay data and identify structure correlations. Supply, through synthesis and procurement, analogs of compounds identified as worthy of further study to newly established bioassays.

b. Other Anastrepha

6. Female Attractants

7. Dispensing Systems

I.B.7 Leonhardt, B. A. CRIS 1275-22000-089-00D

Also: I.D.6

SY: 0.1

Objective: Develop solid controlled-release polymeric dispensers for synthetic attractants to be used in traps to detect the presence of fruit flies such as oriental, Mediterranean, Malaysian and melon flies.

Significance: Such dispensers would be used in detection traps in the U. S. and elsewhere and replace the use of liquid lure applied to cotton wicks. These dispensers would prolong effectiveness, reduce contamination and facilitate baiting traps.

Justification: Industry - I.C; Regulatory - I.A, I.I.

Constraints: The chemical properties of some of the attractants may not be compatible with the polymers and additives now used to make the Trimedlure polymeric

plugs. New formulations may need to be investigated. Large-scale comparisons of the efficacy of the formulated lure as compared to the liquid form would be necessary. Performance criteria (chemical and biological) would need to be established.

References: None.

Co-investigators/Cooperators: R. T. Cunningham, ARS, Hilo; AgriSense, Fresno; R. E. Rice, Univ. of California, Davis. Potential: E. Jang, ARS, Hilo; California Dept. of Food and Agriculture; APHIS S&T.

Work Plans for FY 92: Obtain test dispensers from manufacturers, field age dispensers and measure release rates.

C. Food and Other Attractants

Background (food-based) - Protein hydrolysate food baits are non-specific for the target insect; numerous other fly species are trapped also. When used in liquid form in McPhail traps, borax additive is frequently required, especially to reduce decay of trapped fly specimens. The borax-containing trap contents are a disposal problem in many locations.

Anastrepha spp.: Food baits such as protein hydrolyzate or torula yeast are the only attractants presently available for this group.

Medflies: Corn protein-derived hydrolysates (NLIB) are the attractive ingredient in the bait sprays used to suppress medfly outbreaks.

Background (host plant-based) - Host plant-produced semiochemicals are likely involved in host location by gravid females. Very little is presently known about the degree of qualitative complexity required in a host plant-released volatiles mixture to induce a female fruit fly to approach the host plant.

Needs (food-based) - When protein hydrolysate is used in liquid form in McPhail traps, a non-toxic alternative for borax is needed. If the active constituents of hydrolyzed protein baits could be identified and synthesized, an attractant mixture of known composition might be formulated, which might lend itself to use in dry traps.

Needs (host plant-based) - If host plant-emitted volatile compounds used by female flies in host-finding could be identified, they would potentially be of great value as female

attractants, particularly in the case of mated flies. Examination of plants, taxonomically related to known hosts, for natural products or emissions that are attractive to flies should be conducted.

1. Proteinaceous Food Baits

a. Anastrepha

I.C.1.a Robacker, D.

CRIS 6204-43000-005-01T
(CDFA)

SY: 0.5

Objective: Identify chemicals produced by bacteria associated with Mexican fruit fly that are attractive to the fly and develop a new attractant.

Significance: The attractant could be used to attract the same segment of the population as protein baits but could be used in a simpler trap.

Justification: Industry - I.A; Regulatory - I.A., I.D, I.I., I.O, III.D.

Constraints: Identification and formulation of the chemicals may require development of new technologies.

References:

Robacker, D. C., J. A. Garcia, A. J. Martinez and M. G. Kaufman. 1991. A strain of Staphylococcus attractive to laboratory strain Anastrepha ludens, (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 84: (In press).

Co-investigators/Cooperators: California Department of Food and Agriculture; W. F. Haddon and R. A. Flath, ARS, Albany; R. R. Heath, ARS, Gainesville. Potential: Scentry, Inc. or other companies with expertise in formulations.

Work Plans for FY 92: Identify primary components. Test in lab and greenhouse bioassays.

I.C.1.a Cunningham, R. T.

CRIS 5320-22000-005-00D

Also: I.C.1.b; I.C.1.c

SY: 0.1

Objective: Development of proteinaceous food baits with enhanced attraction through an investigation of their

attractive components and the further development of the oligocomponent, defined food bait substitute.

Significance: Food baits are attractive to both sexes in both the sexually immature and mature states of all tephritids thus far tested. They are the only attractants for general use in the detection trap arrays for the Anastrepha genus, including the Mexican and Caribbean fruit flies. Further, food baits are the vital component of one of the three eradication techniques. In the case of certain exotic Anastrepha and Dacinae, they are the basis for the only eradication techniques available to handle introductions into the continental U.S.

Justification: Industry - I.C, III.A, III.C, III.G;
Regulatory - I.A, I.D, I.O, III.D, III.QQ.

Constraints: None.

References: None.

Co-investigators/Cooperators: R. Teranishi and R. A. Flath, ARS, Albany; N. Wakabayashi, ARS, Beltsville.

Work Plans for FY 92: Initiate field bioassay of attractive components of protein hydrolysate.

Also see Sivinski I.A.2.b, Teranishi, Kint and Light I.H.

b. Mediterranean Fruit Fly

Also see Cunningham I.C.1.a.

c. Bactrocera (Dacus)

Also see Cunningham I.C.1.a, Jang I.H, Liquido and Jang I.H, Teranishi, Kint and Light I.H.

2. Host-derived Attractants

a. Anastrepha

I.C.2.a Robacker, D.

CRIS 6204-43000-005-01T
(CDFA)

SY: 0.4

Objective: Evaluation in the field of the host-fruit-derived attractant for Mexican fruit fly and transfer the technology into commercial production of new lures and

traps to replace proteinaceous baits in McPhail traps as the standard trapping system for this fly.

Significance: Such an attractant would become widely used (worldwide scale) if it was more attractive than proteinaceous baits currently used or if it was as attractive as protein baits and could be used in a simpler trap. Costs for conducting trapping operations would probably decrease and if the new lure was more attractive than currently used baits, costs of eradication would also decrease since infestations could be detected at an earlier stage.

Justification: Industry - I.A; Regulatory - I.A, I.D, I.I, I.O, III.D.

Constraints: None.

References:

Robacker, D. C., A. M. Tarshis Moreno, J. A. Garcia and R. A. Flath. 1990. A novel attractant for Mexican fruit fly, Anastrepha ludens, from fermented host fruit. J. Chem. Ecol. 16:2799-2815.

Co-investigators/Cooperators: California Department of Food and Agriculture (CDFA); R. A. Flath, ARS, Albany; Scentry, Inc., Buckeye, AZ; APHIS S&T, Guatemala.
Potential: R. R. Heath, ARS, Gainesville; Texas Department of Agriculture (TDA); Mexican Agricultural Agencies.

Work Plans for FY 92: One field test each during Autumn 1991 and Spring 1992 of host-fruit-derived attractant vs PIB-7 in Guatemala.

I.C.2.a Heath, R. R.

CRIS 6615-22000-008-07R

Also: I.C.2.b

SY: 0.1

Objective: Develop and improve existing food attractants.

Significance: Increased knowledge of the chemistry associated with protein baits and host chemicals may provide the ability to detect a broader spectrum of the fruit fly population as well as multiple species. Research will be conducted to determine the specific semiochemicals that are produced from yeast hydrolyzates, Nulure, several candidate bacterial cultures and host plants.

Justification: Industry - I.A, II.B, III.G; Regulatory - I.A, I.B, I.D, I.K, I.M, I.O.

Constraints: Development of suitable bioassays.
Synthesis and formulation of chemicals identified as attractive.

References: None.

Co-investigators/Cooperators: P. J. Landolt, J. M. Sivinski, C. O. Calkins and N. D. Epsky, ARS, Gainesville; D. Robacker, ARS, Weslaco; APHIS S&T, Guatemala.

Work Plans for FY 92: Determine what chemical(s) released from protein baits and several candidate host plants may be attractive to Anastrepha.

Also see Sivinski I.A.2.b.

b. Mediterranean Fruit Fly

I.C.2.b Flath, R. A. CRIS 5325-22000-010-00D
Light, D. M.

Also: I.C.2.c

SY: 0.15 (Flath), 0.15 (Light)

Objective: Identification of host plant-based semiochemicals (attractants, arrestants and stimulants) for mated/gravid females of the four Hawaiian fruit fly species. Assess the chemoreceptive basis of how these odors promote, drive and modulate foraging/selection/oviposition behaviors of female fruit flies.

Significance: The ability to monitor female fruit fly populations, sterile and fertile, would be valuable in outbreak detection and eradication efforts. This would be particularly true when employing the sterile insect technique (SIT). At present, no useful female-specific attractants are available. Fruit of several plant species growing in Hawaii, including Jerusalem cherry (Solanum pseudocapsicum) are heavily infested by the medfly. On the U. S. mainland, many commercially-important fruit crops, including the stone fruit, are favored hosts. Host odor is clearly a factor in bringing mated females to host plants. Active components of emissions from such fruit fly host plants might prove useful as female attractants. Knowledge of how fly physiology modulates behavior will enhance our understanding of complex behaviors.

Justification: Industry - I.B; Regulatory - I.A, I.D.

Constraints: The effectiveness of such host-based attractants will depend upon the intensity of the behavioral response elicited in the female by an attractant; whether competing attractant sources are present at levels sufficiently high to cause confusion; and whether other behavioral cues, such as color and shape, are obligatory or merely reinforcing cues in host location by the female.

References:

- Takeoka, Flath, et al. 1988. Nectarine volatiles:... J. Agric. Food Chem. 36:553-560.
- Engel, Flath, et al. 1988. Investigation of volatile constituents in nectarines. 1... J. Agric. Food Chem. 36:549-553.
- Engel, Ramming, Flath, et al. 1988. Investigation of volatile constituents in nectarines. 2... J. Agric. Food Chem. 36:1003-1006.
- Takeoka, Flath, et al. 1990. Volatile constituents of apricot. J. Agric. Food Chem. 38:471-477.
- Buttery, R. G., L. Ling and D. M. Light. 1987. Tomato leaf volatile aroma components. J. Agric. Food Chem. 35:1039-1042.
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- Light, D. M. and E. B. Jang. 1988. Modulation of fruit-foraging behaviors of female Ceratitis capitata through chemoreception of volatiles indicative of different maturation states of nectarines. XVIII Inter. Congr. Entomol. (Abstract) PP. 213.
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- Jang, E. B. and D. M. Light. Behavioral responses of female oriental fruit flies to the odor of papayas at three ripeness stages in a laboratory flight tunnel. J. Insect Behav. (In press).
- Light, D. M., E. B. Jang and R. A. Flath. Electroantennogram responses of the Mediterranean fruit fly, Ceratitis capitata, to the volatile constituents of nectarines. Ent. Exp. Appl. (In press).

Co-investigators/Cooperators: R. T. Cunningham and E. B. Jang, ARS, Hilo.

Work Plans for FY 92: Further examination/testing of J. cherry components. Preparation of stone fruit concentrates for bioassay.

I.C.2.b Warthen, J. D. CRIS 1275-22000-086

SY: 0.1

Objective: The primary host, Coffea robusta, for medflies will be examined for attractants. Hexane, methylene chloride, and ethanol rinses of the berries at

Work Plans for FY 92: Minimal this year. Concentrates prepared for medfly work will be screened for Bactrocera activity also.

I.C.2.c Jang, E. B.

CRIS 5320-22000-005-00D

Also: III.B.2

SY: 0.2

Objective: Development of female attractant for Bactrocera spp.

Significance: Current attractants are effective primarily for males of all species. Although protein baits will attract females, new compounds are needed which are specific for females. Such an attractant would greatly augment current male-based systems allowing for improved control and eradication technology.

Justification: Industry - I.C; Regulatory - I.D, I.O, III.XX, III.DDD.

Constraints: None.

References: None.

Co-investigators/Cooperators: R. A. Flath, ARS, Albany; J. Stark, WSU. Potential: D. Light, ARS, Albany; N. J. Liquido, ARS, Hilo.

Work Plans for FY 92: Bioassay compounds attractive to Bactrocera females. Identify components in cooperation with chemists (Flath).

Also see Flath I.B.4.a, Flath and Light I.C.2.b, Jang I.H.

D. Traps, Devices and Formulations

Background - Concentrated efforts have not been expended in the development of new traps or designs in several years. The discovery by Landolt et al. of the need for visual cues in combination with pheromone for the papaya fruit fly has opened a new way of looking at trap design. Females are attracted to Jackson traps baited with trimedlure but will seldom enter when males are present. The use of polymeric plugs for formulation of trimedlure and the basket it is placed have eliminated some of the problems with the liquid. Techniques for determination of trap densities [for the probabilities of detection] of various population levels have been developed for Caribbean fruit flies.

Needs - a trap that is more efficient in catching female medflies is needed when a sex pheromone is used. Visual cues

and pheromones need to be incorporated into dry traps for species of Anastrepha. Trap densities and placements for greatest probabilities of detection need to be investigated for many species. Effective controlled-release dispensers need to be developed for attractants other than trimedlure. Efforts to develop lures and trapping systems have been concerned primarily with economics, with little research directed towards increasing trap efficiency in order to improve our ability to detect small populations.

1. Mediterranean Fruit Fly

I.D.1 Liquido, N. J.

CRIS 5320-22000-012-00D
5320-22000-005-00D
5320-22000-013-00D

Also: I.D.3; I.D.4; I.D.6

SY: 0.1

Objective: Development of an efficient trapping system for the Mediterranean fruit fly, oriental fruit fly, melon fly and Malaysian fruit fly.

Significance: Information on the optimum trap density and trapping grid design is vital in detecting fruit fly introductions efficiently, in predicting the density of adults per unit area and in timely application of eradication treatments.

Justification: Industry - I.C; Regulatory - I.A, I.H, I.M, I.N, III.GG, III.II.

Constraints: None.

References: None.

Co-investigators/Cooperators: R. Plant, Univ. of California, Davis; APHIS S&T; CDFA.

Work Plans for FY 92: Compare efficiency of all traps (dry and wet) currently being used in fruit fly detection and survey programs; initiate research on optimum trap density and efficient trap array.

2. Anastrepha

I.D.2 Robacker, D.

CRIS 6204-43000-005-01T
(CDFA)

SY: 0.2

Objective: Find a trap for the most promising new attractants that is less expensive and easier to use than McPhail traps.

Significance: The McPhail trap is bulky, fragile and expensive to use but is necessary with protein baits. Replacing the McPhail trap with a better trap will save time and money.

Justification: Industry - I.A; Regulatory - I.A.

Constraints: None.

References:

Robacker, D. C., D. S. Moreno and D. A. Wolfenbarger. 1990. Effects of trap color, height and placement around trees on capture of Mexican fruit flies (Diptera: Tephritidae). J. Econ. Entomol. 83:412-419.

Co-investigators/Cooperators: APHIS S&T, Guatemala; CDFA; Scentry, Inc.; Great Lakes IPM, Vestaburg, MI.
Potential: R. R. Heath, P. J. Landolt, ARS, Gainesville; Texas Department of Agriculture; Mexican Agricultural Agencies.

Work Plans for FY 92: Field test yellow spheres, yellow panels, Jackson traps and novel designs in conjunction with tests of host-derived attractant.

Also see Sivinski I.A.2.b, Heath III.B.2.c.

3. Bactrocera (Dacus)

Also see Liquido I.D.1.

4. **Systems**

I.D.4 Robacker, D.

CRIS 6204-43000-005-01T
(CDFA)

SY: 0.2

Objective: Evaluate combinations of fruit-derived attractants, bacteria-derived attractants and pheromones in appropriate traps as a trapping system for Mexican fruit fly.

Significance: Such an attractant would become widely used (worldwide scale) if it was more attractive than proteinaceous baits currently used or if it was as attractive as protein baits and could be used in a simpler trap. Costs for conducting trapping operations would probably decrease and if the new lure was more

attractive than currently used baits, costs of eradication would also decrease since infestations could be detected at an earlier stage.

Justification: Industry - I.A; Regulatory - I.A, I.D, I.I, I.O, III.D.

Constraints: None.

References:

- Robacker, D. C. and J. A. Garcia. 1990. Responses of laboratory strain Mexican fruit flies, Anastrepha ludens, to combinations of fermenting fruit odor and male-produced pheromone in laboratory bioassays. J. Chem. Ecol 16:2027-2038.
- Robacker, D. C. Specific hunger in Anastrepha ludens (Diptera: Tephritidae): Effects on attractiveness of proteinaceous and fruit-derived lures. Environ. Entomol. (In press).

Co-investigators/Cooperators: Potential: CDFA; R. R. Heath, P. J. Landolt, ARS, Gainesville; APHIS S&T, Guatemala; Mexican Agricultural Agencies; Scentry, Inc. or other companies with expertise in formulations.

Work Plans for FY 92: Lab bioassays of host-fruit-derived attractant/pheromone combination if pheromones are ready early enough during FY 92.

Also see Liquido I.D.1.

5. Trap Locations

6. Attractant Dispensers

Also see Leonhardt I.B.7, Liquido I.D.1.

E. Host Survey Techniques

1. Acoustics

Also see Calkins II.C.2.

F. Systematics

Background - Genetically isolated medfly populations have been distinguished by isozyme analyses, however, similar studies have been much more limited in Anastrepha and Bactrocera species. This information is of particular importance to routine species identification of Anastrepha larvae and adult males caught in traps, which is presently lacking. Molecular genetic methods can provide highly sensitive means to more extensively identify species and strains.

Needs - The ability to determine from what population a specimen or group of specimens came from is important when quarantine or eradication personnel are trying to stop the movement of these invading insects. Mitochondria DNA has been proposed as a more accurate and simpler method of separating populations than the use of isoenzymes. There may be other methods that are in the process of development that could be used for such a purpose. Pteridine concentration in the eye is a good method of adult age determination and should be pursued. A method to determine whether sperm stored in a trapped female came from a sterile or a fertile male is needed to evaluate the effectiveness of the released flies in a sterile release eradication program.

1. Genetic

I.F.1 McInnis, D. CRIS 5320-22000-011-00D

Also: I.F.4

SY: 0.1

Objective: Genetic characterization of tephritid fruit fly populations.

Significance: Basic genetic studies of fruit flies may uncover new chromosomal, isozymic, or molecular level variation useful in developing new forms of powerful genetic control methods. Another application of basic genetic studies might be the discovery of reliable diagnostic variation useful in pinpointing the origins of fruit fly infestations in exotic areas.

Justification: Regulatory - III.EE.

Constraints: None.

References: None.

Co-investigators/Cooperators: D. Haymer, Univ. of Hawaii, Honolulu; M. Zapater, Univ. of Buenos Aires, Argentina.

Work Plans for FY 92: Isozyme variation will continue to be monitored in wild Hawaiian tephritid populations. Collaborative DNA fingerprinting investigations will continue on fly samples from Hawaii, Latin America, and hopefully, California (CDFA preserved samples).

I.F.1 Huettel, M. D. CRIS 1275-22000-008-04R

Also: I.F.4

SY: 0.3

Objective: Develop genetic markers for identifying the Mediterranean fruit fly, Ceratitidis capitata (Wiedemann) to the subpopulation level. Build a data base on the distribution of these markers among the various world populations of this insect. Develop a system for assessing the most probably origin of future invasions of this insect into the U. S.

Enzyme-specific staining of whole fly homogenates separated by starch gel electrophoresis will be used to visualize a randomly chosen set of 15-20 enzymes and their genetic variants. Resulting data will be analyzed using the BIOSYS software. Mitochondrial DNA will be isolated and purified from mass-reared medfly and used to probe the nucleic acids from wild flies. DNA from these flies will be separated by gel electrophoresis and analyzed by Southern blot techniques to identify endonuclease generated fragments (RFLPs).

A transfer plan will be developed for implementation of population fingerprinting technology. At one level, only the skills (knowledge) necessary to interpret data provided by a contractor would be provided. At the second level, both the electrophoretic and interpretive skills would be transferred. In either case, the entire BIOSYS data base will be an integral part of the transfer.

Significance: Knowing the source of flies that have invaded the U.S. is important in determining pathways and identifying possible priorities for control or eradication of out-of-country sources.

Justification: Regulatory - III.EE.

Constraints: Since population "fingerprinting" is a statistical process, it is necessary to have detailed data from all likely sources of introduced medflies. Furthermore, the correlation between inter-population variation in nuclear genes and that in mtDNA RFLPs is unknown for medfly because no world-wide data base exists for the latter.

References: None.

Co-investigators/Cooperators: W. S. Sheppard, ARS, Beltsville; E. W. Quinn/S. Berlocher.

Work Plans for FY 92: Medfly samples from the 1989-90 infestation of Los Angeles Co., California will be analyzed on a priority basis.

I.F.1 Sheppard, W. S. CRIS 1275-21220-024-00D

Also: I.F.4

SY: 0.1

Objective: To study the population genetics of colonization by the medfly. To characterize mitochondrial and nuclear DNA variation in introduced New World and endemic Old World medfly populations with a special interest in identifying source areas of U. S. infestations.

Significance: Accurate knowledge of source areas will help focus interdiction efforts to appropriate channels. Effective quarantine regulations and control tactics against the medfly depends on a thorough knowledge of their distribution, population dynamics and structure.

Justification: Regulatory - III.EE.

Constraints: None.

References:

- Sheppard, W. S., T. E. Rinderer, J. A. Mazzoli, J. A. Stelzer and H. Shimanuki. 1991. Gene flow occurs between African- and European-derived honey bee populations in Argentina. *Nature* 349:782-784.
- Azeredo-Espin, A. M., R. F. W. Shroder, M. D. Huettel and W. S. Sheppard. 1991. Mitochondrial DNA variation in geographic populations of Colorado potato beetle (Coleoptera: Chrysomelidae). *Experientia* 47:483-485.
- Steck, G. J. and W. S. Sheppard. Mitochondrial DNA variation in *Anastrepha*. In *Fruit Flies of Economic Importance*, C. V. Cavallero, Ed. (In press).
- Sheppard, W. S. and B. A. McPheron. Ribosomal DNA diversity in *Apis*. In *Diversity in Apis*. D. R. Smith, Ed. Westview Press, Boulder, CO. (In press).

Co-investigators/Cooperators: G. J. Steck, Florida Department of Agriculture and Consumer Services, DPI, Gainesville, FL; B. A. McPheron, Dept. of Entomology, Pennsylvania State University, CDFA.

Work Plans for FY 92: Continue MtDNA RFLP analysis and mapping of available feral medfly samples, screen medfly populations for nuclear DNA variation using RAPD primers, acquire additional feral medfly samples from Central and South America and Europe, obtain live or frozen samples from any California infestations found during the cropping season, develop PCR primers in informative regions for use with degraded (pinned) medfly samples.

I.F.1 Campbell, B.

CRIS 5325-22000-010-00D

Also: I.F.4

SY: 0.7

Objective: Analysis of mitochondrial and symbiotic 16S rDNAs as a molecular phylogenetic approach to differentiate geographic and host-plant races of tephritids.

Significance: There is a need to determine the geographic origin of a number of tephritid flies of economic importance. Some of these flies have historically showed repeated cases of inceptive outbreaks which have required eradication efforts. Knowing where these flies originated would facilitate developing measures for exclusion. Molecular phylogenetics can be useful in differentiating morphologically indistinguishable species complexes of flies that are pests and that are used in biological control programs.

Justification: Industry - II.C, III.G, III.I; Regulatory - I.C, III.BB, III.NN, III.AAA.

Constraints: Access to quarantined flies caught in traps.

References:

- Campbell, B. C., T. J. Bragg and C. Turner. Phylogeny of symbiotic bacteria of four weevil species (Coleoptera: Curculionidae) based on analysis of 16S ribosomal DNA. *Insect Biochem.* (Submitted)
- Munson, et al. Aphid endosymbiosis: Evidence of a bacterial infection of an ancestor of four aphid families. *J. Bacteriol.* (In press).
- Campbell, B. C. and B. M. Unterman. 1989. Purification of DNA from the intracellular symbiotes of *Sitophilus oryzae* and *Sitophilus zeamais* (Coleoptera: Curculionidae). *Insect Biochem.* 19:85-88.
- Campbell, B. C. 1989. On the role of microbial symbiotes in herbivorous insects. PP 1-44 in *Insect-Plant Interactions*, Vol. I (E. A. Bernays, ed.). CRC Press Inc., Boca Raton, FL.

Co-investigators/Cooperators: T. S. Bragg, ARS, Albany; J. D. Steffen-Campbell and C. Turner, ARS, Albany; D. O. McInnis, ARS, Honolulu; N. Liquido, ARS, Hilo; A. Norrbom, ARS, Washington; APHIS S&T, Guatemala.

Potential: CDFA.

Work Plans for FY 92: Procurement of flies from collaborators in Hawaii, Guatemala and USA. Characterize restriction endonuclease site diversity between fly-strains. Perform nucleotide sequencing, data base analyses and phylogenetic analyses.

Also see Campbell I.F.4.

2. Age ID
3. Sperm ID
4. Taxonomic ID

I.F.4 Campbell, B.

CRIS 5325-22000-010-00D

Also: I.F.1

SY: 0.2

Objective: Use restriction fragment length polymorphisms of ribosomal RNA genes of bacterial symbiotes to categorize host-plant races of tephritids used in biological control (e.g. Urophora sirunaseva and Chaetorellia australis currently used for the control of yellow star thistle).

Significance: Certain species of thistle-feeding tephritids have been released into California for the biological control of yellow star thistle. Some new host-plant races of these flies have become established on other species of thistles. There is currently no method of differentiating these host-plant races of flies.

Justification: Regulatory - III.AAA.

Constraints: Field collection of flies over large geographical area from a number of thistle species throughout a number of seasons; staff limitations.

References:

- Campbell, B. C., T. J. Bragg and C. Turner. Phylogeny of symbiotic bacteria of four weevil species (Coleoptera: Curculionidae) based on analysis of 16S ribosomal DNA. Insect Biochem. (Submitted)
- Munson, et.al. Aphid endosymbiosis: Evidence of a bacterial infection of an ancestor of four aphid families. J. Bacteriol. (In press).
- Campbell, B. C. and B. M. Unterman. 1989. Purification of DNA from the intracellular symbiotes of Sitophilus oryzae and Sitophilus zeamais (Coleoptera: Curculionidae). Insect Biochem. 19:85-88.
- Campbell, B. C. 1989. On the role of microbial symbiotes in herbivorous insects. PP 1-44 in Insect-Plant Interactions, Vol. I (E. A. Bernays, ed.). CRC Press Inc., Boca Raton, FL.

Co-investigators/Cooperators: C. Turner, ARS, Albany.

Potential: K. S. Hagen, Univ. of California, Berkeley.

Work Plans for FY 92: Collection of flies, isolation and purification of genomic DNAs, development of polymerase

chain reaction to amplify 16S rRNA genes, examination of restriction digest of 16S rRNA genes for diagnostic characteristics.

I.F.4 Thompson, F. C.

CRIS 1275-22000-048-00D

CRIS 0500-00001-010-00D

(Funds are pilot test and terminate in FY 92.)

SY: 0.5

Objective: Provide a prototype expert system and biosystematic information database using tephritid flies as an example; demonstrate increased systematic research productivity; and establish benefits of new approaches to synthesis and dissemination of biosystematic information.

Significance: If such a system can become effective and economical, it would probably allow most field workers and quarantine inspectors to perform their own identifications of fruit flies and retrieve the basic biological data on them. Allowing users to do their own identifications would then relieve the demand on the experts who would then have more time to research cryptic species complexes and develop new and better identification systems.

Justification: Industry - II.C; Regulatory - I.C.

Constraints: A convincing demonstration of efficacy requires a prototype that is comprehensive, including all the pest species as well as other commonly encountered species. Such comprehensiveness requires considerable data capture work, especially for images. The expert system requires a complete character X species data matrix, whereas the current literature provides only some characters for all species, but not all characters for all species. So, the critical bottleneck is getting data into the system, and most of that data is only currently accessible to the experts. Funds needed to continue data capture.

References: None.

Co-investigators/Cooperators: A. L. Norrbom, ARS, Washington; I. White, CAB Entomology, London; A. Freidberg, Tel Aviv Univ., Israel. Potential: APHIS S&T at field test stage.

Work Plans for FY 92: Completion of data capture; beta-testing of expert system and biosystematic information database; production of final field version.

I.F.4 Norrbom, A. L.

CRIS 1275-22000-048-00D

SY: 0.2

Objective: Revision of the systematics of various species groups of Anastrepha, including the fraterculus complex, by morphological and biochemical analyses.

Significance: Anastrepha is the largest and most economically important genus of Tephritidae in the Neotropics. The ability to distinguish both pest and non-pest species is critical to detection, quarantine and control programs, as well as many scientific studies involving them.

Justification: Regulatory - I.C; III.NN.

Constraints: Limited technical support (1/3 technician per SY) and operating funds limits amount of field work important for collecting and rearing material needed for analyses. Funds to support foreign collaborators to conduct this work are also limited.

References:

- Norrbom, A. L. 1991. The species of Anastrepha (Diptera: Tephritidae) with a grandis-type wing pattern. Proc. Entomol. Soc. Wash. 93:101-124.
 Norrbom, A. L. and K. C. Kim. 1988. A list of the reported host plants of the species Anastrepha Schiner (Diptera: Tephritidae). U. S. Dept. Agric., Animal and Plant Health Inspection Service, Plant Protection and Quarantine, No. 81-52, Hyattsville, Maryland, 114 pp.

Co-investigators/Cooperators: G. J. Steck, DPI, Gainesville; A. Malavasi and J. Morgante, Sao Paulo, Brazil; R. A. Zucchi, Piracicaba, Brazil.

Work Plans for FY 92: Continued accumulation of specimens and associated data for systematic analysis. Description of several new species. Morphological characterization of some populations of the Anastrepha fraterculus species complex.

I.F.4 Norrbom, A. L.

CRIS 1275-22000-048-00D

(Also supported by grant from USDA, OICD: U. S. - Brazil Science and Technology Initiative, \$15,000 over FY 90 and 91.)

SY: 0.2

Objective: Taxonomic revision of Toxotrypana.

Significance: Toxotrypana includes at least 10 species, two of which are pests of Carica. Several species, some of which are undescribed, are difficult to distinguish from the "papaya fruit fly" (T. curvicauda), leading to confusion about its distribution. The distributions and hosts of all of the species are poorly known. Revision of their taxonomy will permit identification and aid the study of their biology, behavior and control.

Justification: Regulatory III.NN.

Constraints: Very careful field studies and rearing are needed in South America to determine if several nominal species are distinct or just color morphs of the same species. The difficulty of encountering some of them in the field has limited the possibility for biochemical analyses.

References: None.

Co-investigators/Cooperators: R. A. Zucchi, Piracicaba, Brazil; G. J. Steck, DPI, Gainesville; P. J. Landolt, ARS, Gainesville.

Work Plans for FY 92: Continued accumulation of specimens and associated data for systematic analysis. Complete morphological revision of Toxotrypana curvicauda.

I.F.4 Norrbom, A. L.

CRIS 1275-22000-048-00D

SY: 0.2

Objective: Production of Handbook of the Tephritidae of Mexico and Guatemala.

Significance: Mexico is one of the largest trading partners of the United States, and the fruit flies of this region will become even more significant to U. S. agriculture if a free trade agreement is negotiated. MesoAmerica is also one of the centers of diversity of the Asteraceae and many composite-breeding tephritids potentially useful as biocontrol agents occur there. Determining what species are present and providing a means to identify them, and a summary of their distributions and hosts are the major aims of the book.

Justification: Regulatory - I.C; III.NN.

Constraints: This is a long-term project that will not be completed in the next 5 years.

References: None.

Co-investigators/Cooperators: V. Hernandez, Instituto de Ecologia, Xalapa, Mexico; Programa MoscaMed, Tapachula, Mexico.

Work Plans for FY 92: Continued accumulation of specimens and associated data for systematic analysis. Complete revision of Mexican species of Trypeta (with H.-Y. Han).

I.F.4 Foote, R. H. CRIS 1275-22000-048-00D
Norrbom, A. L.

SY: 0.05

Objective: Handbook of the Fruit Flies (Diptera: Tephritidae) of America North of Mexico.

Significance and Justification: To be able to detect and exclude foreign fruit flies, we must know how to recognize the native and previously introduced species, which is the major aim of this handbook by R. H. Foote, F. L. Blanc, and A. L. Norrbom. Distribution, host and other biological data are included. The manuscript is in final editing and should be published in FY 92 by Cornell University Press.

Constraints: None.

Co-investigators/Cooperators: F. L. Blanc, CDFA, retired.

References: None.

Work Plans for FY 92: Final edition and publication.

I.F.4 Leonhardt, B. A. CRIS 1275-22000-089-00D

SY: 0.1

Objective: Explore the possibility of using cuticular hydrocarbons to identify source of medflies. Use gas chromatography and mass spectrometry to identify hydrocarbon profiles on solvent extracts of flies.

Significance: The identification of the geographic origin of introduced medflies will help determine the source of the flies so that preventive measures can be increased. Hydrocarbon profiles have been used to discriminate other insect species and may be useful for fruit flies as well.

Justification: Regulatory - I.C.

Constraints: None.

References: None.

Co-investigators/Cooperators: R. T. Cunningham, ARS, Hilo; APHIS S&T, Guatemala.

Work Plans for FY 92: Compare hydrocarbon profiles of male and female medflies from Hawaii and Guatemala. Compare profiles of wild and lab-reared flies.

Also see McInnis I.F.1, Huettel I.F.1, Campbell I.F.1, Sheppard I.F.1.

G. Semiochemical Structure/Activity Relationships

Background - Little is known about how attractants interact with olfactory receptors to elicit specific behaviors such as attractancy. Structure/activity relationships are key studies to understanding how attractants work. Positive correlations have been made between electrophysiology and behavior of medflies to trimedlure isomers. Similar behavioral assays have led to the development of Ceralure, a more persistent but equally attractive analog to trimedlure.

Needs - Close interaction between synthetic chemists and insect neurophysiologists are needed to develop suitable techniques and assays to study structure/activity relationships. We must broaden our understanding of how the numerous sensory modalities in fruit flies are integrated in the CNS leading to behavioral responses. Analogs of current attractants should be tested as available for structure/activity.

*I.G Jang, E.
Light, D.*

*CRIS 5325-22000-010-00D
5320-22000-005-00D*

SY: 0.1 (Jang), 0.1 (Light)

Objective: Improvement of current attractants through studies of structure/activity between chemicals and olfactory receptors responsible for fly behavior.

Significance: The potential improvement of current attractants through knowledge of structure/activity relationships could result in a new class of "designer attractants" which would be more attractive or persistent than the parent compound.

Justification: Industry - II.C, III.H; Regulatory - I.D, I.F, I.I.

Constraints: Electrophysiological techniques for recording individual olfactory receptors in fruit flies are difficult due to the small size of the receptors. Improved equipment and techniques are needed to facilitate cooperation with synthetic chemists.

References:

- Light, D. M. and E. B. Jang. 1987. Electroantennogram responses of the Oriental fruit fly, Dacus dorsalis, to a spectrum of alcohol and aldehyde plant volatiles. Entomol. Exp. Appl. 45:55-64.
- Light, D. M., E. B. Jang and J. C. Dickens. 1988. Electroantennogram responses of the Mediterranean fruit fly, Ceratitis capitata to a spectrum of plant volatiles. J. Chem. Ecol. 14:159-180.
- Jang, E. B., D. M. Light, R. A. Flath, J. T. Nagata and T. R. Mon. 1988. Electroantennogram responses of the Mediterranean fruit fly, Ceratitis capitata (Wiedmann) to isolated and identified volatile constituents from males. XVIII Intl. Cong. Entomol. (Abstract). P. 138.
- Jang, E. B., R. A. Flath, J. T. Nagata and T. R. Mon. 1989. Electroantennogram responses of the Mediterranean fruit fly, Ceratitis capitata to identified volatile constituents from calling males. Entomol. Exp. Appl. 50:7-19.
- Jang, E. B., D. M. Light, J. C. Dickens, T. P. McGovern and J. T. Nagata. 1989. Electroantennogram responses of the Mediterranean fruit fly, Ceratitis capitata, to trimedlure and its trans isomers. J. Chem. Ecol. 15:2219-2231.

Co-investigators/Cooperators: J. Dickens, ARS, Starkville.
Potential: A. DeMilo, J. D. Warthen, ARS, Beltsville; M. Bengtsson, Lund, Sweden.

Work Plans for FY 92: Develop techniques for recording electrophysiological responses of fruit flies to behaviorally important semiochemicals. Continue EAG studies to host odors, pheromones, para-pheromones, etc.

I.G Mayer, M. S.

CRIS 6615-22000-009-00D

SY: 0.1

Objective: To correlate behavioral responses to pheromones with electroantennogram (EAG) measurements of antennal receptor neurons in Anastrepha spp.

Significance: It is sometimes difficult to determine if a fly has detected a particular olfactory stimulant and failed to respond because it is an ineffective stimulant or whether the stimulant has not been detected. The use of combined behavioral and EAG assays of pheromones and other stimulants will enable investigators to ascertain whether the fly has actually detected the stimulant.

Justification: Industry - I.A, I.B, III.C, III.G;
Regulatory - I.A, I.D, I.I., I.K, I.M, I.O, III.B.

Constraints: The EAG must be employed as an adjunct to behavioral assays--it is not a "stand-alone" assay. Furthermore, for the EAG to have significance, the stimuli must generally be the ones assayed by behavior.

References: None.

Co-investigators/Cooperators: C. Calkins and J. Sivinski, ARS, Gainesville.

Work Plans for FY 92: Electroantennogram recordings of bulk antennal olfactory receptor neuron responses to various odorants such as pheromones, feeding stimulants, etc., will be made in conjunction with behavioral assays.

I.G Warthen, J. D.

CRIS 1275-22000-086

SY: 0.1

Objective: To investigate the structure-activity relationship between attractive synthetic trimedlure and ceralure isomers vs the natural occurring attractant, α -copaene, via computer molecular modeling. Synthesize α -copaene analogs.

Significance: α -Copaene produces greater attraction than ceralure or trimedlure, but is too difficult to synthesize or isolate. An inexpensive analog would probably have great utility in an eradication/detection program.

Justification: Industry - I.B; Regulatory - I.D, I.G.

Constraints: None.

References: None.

Co-investigators/Cooperators: R. T. Cunningham, ARS, Hilo; R. A. Flath, ARS, Albany; A. B. DeMilo, ARS, Beltsville.

Potential: W. Schmidt, Beltsville.

Work Plans for FY 92: Model copaene and develop model of covariance with trimedlure and ceralure.

Also see Warthen I.B.1.a, Warthen I.B.1.b, DeMilo I.B.1.c.

H. Behavior

I.H Robacker, D.

CRIS 6204-43000-005-00D

SY: 0.4

Objective: Study bacteria relationships with wild Mexican fruit flies.

Significance: These bacteria may produce highly attractive chemicals or hold the key to novel approaches to fruit fly control

Justification: Industry - III.E; Regulatory - I.O; III.B, III.DDD.

Constraints: None known at this time.

References: None.

Co-investigators/Cooperators: APHIS S&T, Mission, TX.

Potential: R. R. Heath, ARS, Gainesville; CDFA.

Work Plans for FY 92: Identify bacteria species from wild flies and determine which ones are attractive to flies.

I.H Landolt, P. J.

CRIS 6615-22000-009-00D

Also: IV.A.4

SY: 0.05

Objective: Investigation of tephritid orientation responses to host fruit and elucidation of chemical and visual stimuli important to host location and host selection behavior.

Significance: Knowledge of tephritid host-location behavior may provide novel technology to attract pest species and should assist ongoing efforts to understand male-mating strategies involving female resources.

Justification: Regulatory - I.A, I.D.

Constraints: None.

References: None.

Co-investigators/Cooperators: R. Heath and J. Sivinski, ARS, Gainesville.

Work Plans for FY 92: Develop bioassays and isolate papaya chemicals attractive to papaya fruit flies.

I.H Jang, E.

CRIS 5320-22000-005-00D

Also: I.C.1.c; I.C.2.b; I.C.2.c; III.D.2.e.0.1; IV.B.2; IV.B.5.a; IV.B.6

SY: 0.1

Objective: Identify semiochemicals from host plants and potential food sources (e.g., protein) attractive to fruit flies and their parasites. Assess how these odors drive fruit fly behavior and the physiological factors which modulate behavior.

Significance: Improved technology for detection and eradication programs are needed to maintain quarantine against fruit flies in areas where they currently do not exist. Identification of novel semiochemicals and associated behaviors may uncover new attractants based on the fly's need for food or oviposition substrates. Knowledge of how fruit fly physiology modulates behavior will enhance our understanding of complex behaviors.

Justification: Industry - I.B, II.C, III.H; Regulatory - I.A, I.D, I.H, I.J, I.O, III.DDD, III.EEE.

Constraints: None.

References:

- Light, D. M. and E. B. Jang. 1988. Modulation of fruit-foraging behaviors of female Ceratitis capitata through chemoreception of volatiles indicative of different maturation states of nectarines. XVIII Intl. Cong. Entomol. (Abstract) P. 213.
- Jang, E. B. 1990. Effects of mating on olfactory behavior of female medfly to male pheromone or host odors. In: Proc. Intl. Symp. Fruit Flies of Econ. Importance. Antigua, Guatemala.
- Jang, E. B. 1990. Physiological and biochemical approaches in the development of technology for eradication of fruit fly pests. In: Proc. Symp. on Eradicating Alien Fruit Flies from the Unique Hawaiian Environment. Poipu, Kauai.
- Jang, E. B. and K. A. Nishijima. 1990. Identification and attractancy of bacteria associated with Dacus dorsalis (Diptera:Tephritidae). Environ. Entomol. 19:1726-1731.
- Flath, R. A., D. M. Light, E. B. Jang, T. R. Mon and J. O. John. 1990. Headspace examination of volatile emissions from ripening papaya (Carica papaya L. solo variety). J. Agric. Food Chem. 38:1060-1063.
- Light, D. M. and E. B. Jang. 1987. Electroantennogram responses of the Oriental fruit fly, Dacus dorsalis, to a spectrum of alcohol and aldehyde plant volatiles. Entomol. Exp. Appl. 45:55-64.
- Light, D. M., E. B. Jang and J. C. Dickens. 1988. Electroantennogram responses of the Mediterranean fruit fly, Ceratitis capitata to a spectrum of plant volatiles. J. Chem. Ecol. 14:159-180.
- Jang, E. B. and D. M. Light. 1991. Behavioral responses of the Oriental fruit flies to the odor of papayas at three different ripeness stages in a laboratory flight tunnel. J. Insect Behavior (In press).

Co-investigators/Cooperators: D. M. Light, R. A. Flath, R. Teranishi, ARS, Albany; N. Liquido, ARS, Hilo. Potential: J. D. Warthen, Jr., ARS, Beltsville; APHIS S&T; CDFA.

Work Plans for FY 92: I.D. host plant volatiles and food sources which influence fruit fly behavior; assess physiological factors involved in behavior.

**I.H Liquido, N. J.
Jang, E. B.**

CRIS 5320-22000-005-00D

Also: I.C.1.c

SY: 0.1 (Liquido), 0.1 (Jang)

Objective: Development of proteinaceous food baits with enhanced attractiveness to fruit fly adults. Identify semiochemicals from food substrates that function as attractants, arrestants and stimulants for foraging, selection, ingestion behavior. Assess the chemoreceptive basis of how these compounds influence behavior.

Significance: Proteinaceous food baits are attractive to sexually immature and mature tephritid fruit fly adults of both sexes. Thus, food (protein) bait is the vital component of one of the three technologies for eradicating fruit fly populations. In Anastrepha and some genera of Dacinae, protein bait (in combination with known toxicant[s]) is the only available technology to eradicate accidental introductions into mainland U.S.

Justification: Industry - I.C, III.A, III.C, III.G;
Regulatory - I.A, I.D, I.O, III.D, III.QQ.

Constraints: None.

References: None.

Co-investigators/Cooperators: D. M. Light, ARS, Albany; R. Teranishi and R. A. Flath, ARS, Albany; N. Wakabayashi, ARS, Beltsville.

Work Plans for FY 92: Initiate field bioassay of attractive components of protein hydrolysate.

*I.H Teranishi, R.
Kint, S.
Light, D. M.*

CRIS 5325-22000-009-00D

Also: I.C.1.a; I.C.1.c

SY: 0.5 (Teranishi), 0.5 (Kint), 0.05 (Light)

Objective: Development of proteinaceous food baits with enhanced attraction; investigation of their attractive components. Assess the chemoreceptive basis of how these odors promote, drive and modulate foraging/selection ingestion behaviors of fruit flies.

Significance: Food baits are attractive to both sexes in sexually immature and mature adult states of all tephritids tested so far. Food baits are the basis for the only eradication techniques available in the continental U.S.

Justification: Industry - I.B, III.B; Regulatory - I.D, I.E, I.F, I.O, III.D, III.N, III.DDD.

Constraints: None.

References:

- Matsumoto, K. E., R. A. Flath, T. R. Mon and R. Teranishi. 1985. Protein hydrolysate volatiles as insect attractants. In: Bioregulators for Pest Control, ACS Symp. Series No. 276, P. A. Hedin, Editor, American Chemical Society, Washington, DC. PP 353-366.
- Teranishi, R., R. G. Buttery and T. R. Mon. 1985. Steam distillation-extraction isolation of insect attractive volatiles from protein hydrolyzates. In: Semiochemistry: Flavors and Pheromones, T. E. Acree and D. M. Soderlund, Eds. Walter de Gruyter & Co., Berlin. PP. 169-180.
- Teranishi, R., R. G. Buttery, K. E. Matsumoto, D. J. Stern, R. T. Cunningham and S. Gothilf. 1987. Recent developments in chemical attractants for tephritid fruit flies. In: Alleochemicals Role in Agriculture and Forestry, ACS Symp. Series No. 330, G. R. Waller, Editor, American Chemical Society, Washington, DC. PP 431-438.
- Flath, R. A., K. E. Matsumoto, R. G. Binder, R. T. Cunningham and T. R. Mon. 1989. Effect of pH on the volatiles of hydrolyzed protein insect baits. J. Agric. Food Chem. 37:814-819.

Co-investigators/Cooperators: R. T. Cunningham, E. Jang and N. J. Liquido, ARS, Hilo; D. Robacker, ARS, Weslaco; APHIS S&T, Guatemala.

Work Plans for FY 92: Investigate volatiles from protein hydrolysates.

I.H Landolt, P. J.

CRIS 6615-22000-009-00D

Also: IV.A.2; IV.A.4

SY: 0.05

Objective: Investigation of host and food effects on fruit fly sexual behavior, including selection of calling sites, stimulation of pheromone release, and synergism of attraction responses.

Significance: The determination of such effects and elucidation of their roles in fly efforts to locate mates and resources will aid studies to design lures and traps as well as determination of trapping or annihilation strategies. A direct result could be more powerful chemical lures using combinations of attractants.

Justification: Regulatory - I.A, I.D.

Constraints: None.

References: None.

Co-investigators/Cooperators: R. Heath, ARS, Gainesville;
APHIS S&T, Guatemala.

Work Plans for FY 92: Test for host and food odor synergism
of female response to male pheromone using flight tunnel.

II. Exclusion

A. Quarantine Security

1. Post-Harvest Techniques

Background - Following the withdrawal of ethylene dibromide as a fumigant for fresh fruit, many tropical commodities that are hosts of fruit flies in the genera Bactrocera, Anastrepha and Ceratitidis were no longer available for fruit disinfection. Research approaches have included alternative fumigants, principally methyl bromide, storage conditions such as cold storage and storage in modified atmospheres and temperature treatments, especially hot air, hot water and vapor heat.

Needs - Single, non-residue producing treatments for tropical tephritids are being developed for most large volume tropical fruits such as citrus, Latin American mangos and papayas. Other hosts such as guavas, atemoya, Hawaiian mangos, and Mexican avocados are not amenable to available treatments or are hosts for pests (seed weevils, chalcids) which can tolerate these treatments at maximum, non-phytotoxic levels. Research including fruit fly susceptibility to treatments and host fruit tolerance of treatments needs to be integrated with research on other quarantine pests, post-harvest plant pathology and consumer acceptance.

II.A.1 *Armstrong, J.*

CRIS 5320-43000-009-00D

SY: 1.5

Objective: Develop quarantine treatments for five major new fruits of export importance in Hawaii (atemoya, carambola, litchi, mango, and rambutan). This research was requested by the Hawaii Tropical Fruit Growers' Association and the University of Hawaii. Atemoya and mango have secondary quarantine insect pests, the atemoya seed chalcid and the mango weevil, respectively, for which collateral quarantine treatments must be developed. Develop quarantine treatments for other Hawaii-grown fruits of economic importance.

Significance: These are major fruit crops of export potential from Hawaii that cannot be marketed on the U. S. mainland or in Japan without quarantine treatments for fruit flies. Opening markets for these fruits will expand and diversify Hawaii's agriculture and the state economic base. Successful quarantine treatments for

fruits exported to Japan and other foreign countries impacts balance of trade.

Justification: Industry - II.D; Regulatory - II.A.

Constraints: Fruit availability and tolerance to available treatment technologies.

References:

- Armstrong, J. W. 1982. The development of a hot water immersion quarantine treatment for Hawaii grown Brazilian bananas. *J. Econ. Entomol.* 75(5):787-790.
- Armstrong, J. W. and D. L. Garcia. 1985. Methyl bromide quarantine fumigations for Hawaii-grown cucumbers infested with melon fly and oriental fruit fly. *J. Econ. Entomol.* 78(6):1308-1310.
- Couey, H. M., J. W. Armstrong, J. W. Hylin, W. Thornsburg, A. N. Nakamura, E. S. Linse, J. Ogata, and R. Vetro. 1985. Quarantine procedure for Hawaii papaya, using a hot-water treatment and high temperature, low-dose ethylene dibromide fumigation. *J. Econ. Entomol.* 78:879-884.
- Hansen, J. D., J. W. Armstrong and S. A. Brown. 1989. The distribution and biological observations of the mango weevil, Cryptorhynchus mangiferae (Coleoptera: Curculionidae), in Hawaii. *Proc. Hawaiian Entomol. Soc.* 29:31-39.
- Hansen, J. D. and J. W. Armstrong. 1990. The failure of field sanitation to reduce infestation by the mango weevil, Cryptorhynchus mangiferae (Coleoptera: Curculionidae). *Trop. Pest Mgmt.* 36(4)::359-361.
- Armstrong, J. W., J. D. Hansen, B. K. S. Hu and S. A. Brown. 1989. High-temperature quarantine treatment for papayas infested with tephritid fruit flies (Diptera: Tephritidae). *J. Econ. Entomol.* 82:1667-1674.
- Hansen, J. D., J. W. Armstrong, B. K. S. Hu and S. A. Brown. 1990. Thermal death of oriental fruit fly (Diptera: Tephritidae) third instars in developing quarantine treatments for papayas. *J. Econ. Entomol.* 83(1):160-167.

Co-investigators/Cooperators: R. Paull and C. Cavaletto, Univ. of Hawaii; Hawaii Tropical Fruit Growers' Association, Hawaii Tropical Fruit Growing and Export Industry.

Work Plans for FY 92: Complete carambola refrigeration treatment efficacy data; complete litchi refrigeration treatment efficacy data; complete litchi hot-water immersion efficacy data; continue to develop mango high-temperature forced-air treatment efficacy data; continue to study potential treatments for atemoya and rambutan.

II.A.1 Armstrong, J. CRIS 5320-43000-009-00D

Also: II.A.2.c

SY: 1.5

Objective: Develop quarantine treatments for host commodities of the Malaysian fruit fly, Bactrocera latifrons (Hendel), which include cultivars of eggplant, peppers, tomatoes, and zucchini and other squashes.

Significance: The Malaysian fruit fly has become established on all Hawaiian islands producing fruits of economic and export importance. The development of mass rearing technology for this species should provide for adequate numbers of adults for quarantine treatments research in 1991. When enough adults are available for oviposition response tests, egg collection, or to obtain larval instars, all work with Hawaii-grown exported fruits for which quarantine treatments were developed against Mediterranean fruit fly, melon fly, and/or oriental fruit fly must be done again using Malaysian fruit fly. Furthermore, immediate priority must be given to the development of quarantine treatments for cultivars of peppers and tomatoes, eggplant, zucchini and other squashes, and other economically important hosts of the Malaysian fruit fly so that quarantine treatments are available in the event of a Malaysian fruit fly infestation in California.

Justification: Industry - II.G, II.H; Regulatory - II.A.

Constraints: Availability of Malaysian fruit flies for tests; fruit availability and tolerance to available treatment technologies.

References: None.

Co-investigators/Cooperators: Potential: University of Hawaii, California Department of Food and Agriculture, impacted grower groups.

Work Plans for FY 92: Initiate thermal mortality tests using heat or cold against Malaysian fruit fly eggs and larvae to determine parameters of times and temperatures required to develop potential quarantine treatments. Determine location of eggs and larvae in infested pepper cvs.

II.A.1 Hallman, G.

CRIS 6631-43000-005-00D

SY: 0.1

Objective: Investigate quarantine treatments for minor fruits infested with Caribbean fruit fly.

Significance: Minor fruits, such as canistel, white sapote, sapodilla, sugar apple, and atemoya, are considered hosts of the Caribbean fruit fly, and, as such, cannot be shipped to quarantined areas. Development of quarantine treatments for these commodities would open new markets.

Justification: Industry - II.G; Regulatory II.A.

Constraints: None.

References: None.

Co-investigators/Cooperators: None.

Work Plans for FY 92: Infest fruits with Caribbean fruit fly and subject the fruits to various quarantine treatments.

a. Chemical

II.A.1.a Hallman, G.

CRIS 6631-43000-005-00D

SY: 0.1

Objective: Development of a methyl bromide fumigation quarantine treatment for guavas infested with the Caribbean fruit fly.

Significance: Guavas cannot currently be shipped from Florida to any location that quarantines against the Caribbean fruit fly. Development of a quarantine treatment for guavas will open new markets for that commodity.

Justification: Industry - II.G; Regulatory II.A.

Constraints: None.

References: None.

Co-investigators/Cooperators: J. R. King, ARS, Miami.

Work Plans for FY 92: Infest guavas with Caribbean fruit fly and subject fruits to various cxtime combinations for Probit 9.

0.1 Alternates for Methyl bromide

ARS has no candidate chemical fumigants as replacements. If other agencies or the private sector identify or produce new fumigants, ARS will perform needed research.

b. Physical

II.A.1.b Hallman, G.

CRIS 6631-43000-005-00D

SY: 0.4

Objective: Investigate the application of fruit coatings to quarantine treatments.

Significance: Fruit coatings are being investigated to prolong the storage life of fruits and vegetables. They have been found to reduce survival of fruit fly immatures within the fruits. Also, some quarantine treatments damage fruits, and coating may help reduce the damage.

Justification: Industry - II.H; Regulatory - II.E.

Constraints: None.

References: None.

Co-investigators/Cooperators: M. Nisperos-Carriedo and E. A. Baldwin, ARS, Winter Haven.

Work Plans for FY 92: Infest fruit with Caribbean fruit fly, coat fruits, try different quarantine treatments to determine stability of coatings and insect mortality.

II.A.1.b Jang, E.

CRIS 5320-43000-002-00D

SY: 0.1

Objective: Develop further efficacy data on the effects of shrinkwrap films on disinfestation of fruit fly from papaya and other tropical and temperate fruits. Integrate into systems approach.

Significance: Shrinkwrap has been found to be effective against fruit flies (medfly, melon, oriental) in laboratory studies. Shrinkwrap films are currently used for postharvest shelflife extension for fruits and vegetables and is non-toxic with little or no adverse effects on the commodity.

Justification: Industry - II.C; II.G, II.H; Regulatory - II.A, II.E.

Constraints: Industry must provide proper wrapping machinery for shrinkwrapping fruits. Protocols for compliance are needed. Effects on fruit quality need further studies.

References: None.

Co-investigators/Cooperators: University of Idaho; PIP.

Work Plans for FY 92: Continue quality evaluations of shrinkwrapped fruits. Start large scale efficacy tests to show security.

c. Heat

0.1 Disinfestation

II.A.1.c.0.1 Gould, W. CRIS 6631-43000-005-00D

SY: 0.2

Objective: Develop hot water treatment of guavas into a feasible quarantine treatment. Conduct a large scale test and phytotoxicity work on fruit.

Significance: Currently, no quarantine treatment is available for guavas which therefore may not be shipped to California or other southern states. A useable treatment might be extended to other species of flies present in guavas in other locations.

Justification: Industry - II.G; Regulatory - II.A, II.E.

Constraints: The supply of guavas for research is often limited since growers would rather sell them than donate them for research. Purchasing fruit would require approximately \$5,000. Fruit supply is often inadequate or all of the fruit ripens at the same time due to weather synchronization. This requires the work to be accomplished in a very short period during which the fruit is very abundant.

References: None.

Co-investigators/Cooperators: G. Hallman, ARS, Miami.

Work Plans for FY 92: The hot water immersion quarantine treatment for guavas should be finished by the end of FY 92.

II.A.1.c.0.1 Hallman, G. CRIS 6631-43000-005-00D

SY: 0.1

Objective: Investigate effects of varying factors on the lethality of temperatures used in quarantine treatments to Caribbean fruit fly immatures.

Significance: Models are being developed to relate the thermodynamics of heating and cooling quarantine treatments to mortality in fruit flies. Many factors,

such as rearing temperature, host, genotype of fly, treatment medium, and rearing density, may alter the susceptibility of the flies to temperatures. These factors must be identified, quantified, and included in the models.

Justification: Industry - II.G; Regulatory - II.A.

Constraints: None.

References: None.

Co-investigators/Cooperators: None.

Work Plans for FY 92: Infest guavas and subject the guavas to different times and temperatures.

II.A.1.c.0.1 Armstrong, J. CRIS 5320-43000-009-00D

SY: 1.5

Objective: Complete thermal mortality models for Malaysian fruit fly, Mediterranean fruit fly, melon fly, and oriental fruit fly eggs and larvae under transient temperature conditions that simulate transient fruit temperatures during heat treatment conditions. Similarly, complete cold mortality models for the four fruit fly species.

Significance: Completion of research in progress. These models will facilitate the rapid determination from preliminary tests whether new candidate fruits will tolerate heat or cold treatments.

Justification: Industry - II.G; Regulatory - II.A, II.E.

Constraints: None.

References: None.

Co-investigators/Cooperators: E. B. Jang, ARS, Hilo; C. E. Hayes, Univ. of Hawaii.

Work Plans for FY 92: Complete initial thermal mortality models for Mediterranean fruit fly, melon fly and oriental fruit fly treated with heat or cold; continue thermal mortality studies with Malaysian fruit fly.

II.A.1.c.0.1 Sharp, J. L. CRIS 6631-43000-005-00D

SY: 0.4

Objective: Develop a hot air treatment against A. suspensa in mangoes and grapefruit.

Significance: Alternative quarantine treatments should be developed for various commodities. Methyl bromide is under review and could be rescinded. Methyl bromide and refrigeration cause phytotoxicity.

Justification: Industry - II.G; Regulatory - II.A.

Constraints: None.

References:

- Sharp, J. L. 1989. Preliminary investigation using hot air to disinfest grapefruit of Caribbean fruit fly immatures. Proc. Fla. State Hort. Soc. 102:157-159.
- Sharp, J. L. 1989. Current research on the hot water dip and viable alternatives, and future quarantine research and alternative treatment certification. In: A report on Seminar on quarantine Treatments for Fresh Tropical Fruits Entering the United States of America. Private Sector Relations, Office of International Cooperation and Development, U. S. Department of Agriculture and Regional Office of Central American Programs, July 10-14, 1989. Mazatlan, Mexico.
- Sharp, J. L., J. J. Gaffney, J. I. Moss, and W. P. Gould. 1990. Hot air device for quarantine research. J. Econ. Entomol. 84:520-527.

Co-investigators/Cooperators: Florida Citrus Commission, Lakeland, FL, Brooks and Sons, Inc., Homestead, FL.

Work Plans for FY 92: Infest fruits with Caribbean fruit fly. Subject to treatment with hot air to develop quarantine treatment times and temperatures.

II.A.1.c.0.1 Hansen, J. D. CRIS 6631-43000-005-00D

SY: 0.2

Objective: Development of a quarantine heat treatment against the papaya fruit fly in papayas.

Significance: With a quarantine treatment, papayas grown in Florida, the Caribbean, and Central America can be exported to California, Japan and other locations that have quarantine restrictions. Because these papayas represent a wide variety of phenotypes, new markets would be established that do not compete with Hawaiian-grown cultivars.

Justification: Industry - II.E., II.G; Regulatory - II.A.

Constraints: Although quarantine treatments against fruit flies are available for Hawaiian papayas, the biology and behavior of these pests differ significantly

from those of the papaya fruit fly. Hence, these treatments probably are not effective against the papaya fruit fly. A potential treatment must be useable with a greater assortment of papaya sizes, shapes and cultivars than those grown in Hawaii.

References: None.

Co-investigators/Cooperators: J. E. Peña, University of Florida, Homestead.

Work Plans for FY 92: Test efficacy of current quarantine treatments for other tephritids against the papaya fruit fly. Both forced hot air and hot water baths will be examined. Phytotoxic measurements will be taken on papayas.

II.A.1.c.0.1 Mangan, R. CRIS 6204-43000-005

SY: 190.4

Objective: Develop postharvest quarantine treatments for citrus, mangos and other subtropical and tropical fruits. Develop treatments for new fruits for the Rio Grande Valley such as peaches.

Significance: Treatments are required for importation of fruit from fruit fly infested regions. A backup disinfestation system is needed for areas or periods of time when population suppression systems do not control pests. Treatments using non-chemical methods are desirable to replace fumigants which have been or may be withdrawn for use on fresh produce. Phytotoxicity problems may appear under commercial conditions.

Justification: Industry - II.G, II.H; Regulatory - II.A, II.E.

Constraints: Large numbers of insects must be reared or collected. Fruit flies of interest (A. ludens, A. obliqua) are quarantined by APHIS and must be maintained in approved facility. ARS currently has no engineering staff assigned to this project.

References: None.

Co-investigators/Cooperators: J. Armstrong, ARS, Hilo; J. Sharp, ARS, Miami; K. Shelly, ARS, Weslaco; North American Mango Importers; Texas Citrus Mutual; FoodPro Int'l.; SEMCO.

Work Plans for FY 92: Submit data and report to APHIS for forced hot air treatment of mangos and grapefruit to establish certification standards. Complete lab tests and confirmatory tests for forced hot air treatment of tangerines. Determine infestation methods and begin treatment development for peaches.

II.A.1.c.0.1 Hallman, G. CRIS 6631-43000-005-00D

SY: 0.1

Objective: Investigate modifications of probit for analysis of heat mortality data of Caribbean fruit fly in fruits.

Significance: Fit of mortality of fruit flies inside fruits due to heat treatments often does not fit probit, resulting in inaccurate estimates of the dose of heat required to achieve quarantine security. This may be because there is an added source of variation affecting the results besides the normal distribution upon which probit is based. The depth larvae are inside of fruit is inversely proportionate to the amount of heat they receive. The distribution of this depth could be mixed with the normal (probit) distribution to give a possible more accurate estimate of the dose required to achieve quarantine security.

Justification: Industry - II.G; Regulatory - II.A.

Constraints: None.

References: None.

Co-investigators/Cooperators: Potential: V. Chew, ARS, Gainesville.

Work Plans for FY 92: Obtain data on depth distribution of Caribbean fruit fly larvae in selected fruits, analyze mortality data using depth data. Cooperate with Victor Chew on analysis.

0.2 Phytotoxicity

II.A.1.c.0.2 Chan, H. CRIS 5320-22000-011-00D

SY: 0.5

Objective: Continue further research on the effects of heat on biochemical systems in heat treated commodities using in vivo and in vitro systems with specific attention to its respiratory metabolism, and ripening

enzymes for the purposes of increasing fruit tolerance to heat.

Significance: Heat treatments have been demonstrated to be a feasible alternative quarantine treatment. However, the heat treatments have shown deleterious effects on fruit quality and disease resistance. Further refinements on heat treatments require an increase of knowledge on the mechanisms of heat damage and development of methods for quantitating and assessing the threshold limits of irreversible heat damage.

Justification: Industry - II.G; Regulatory - II.A, II.E.

Constraints: Knowledge and expertise on heat transfer and thermal diffusivity is lacking at this location. An ARS engineer could provide needed consultative services.

References:

- Chan, H. T., Jr., S. Y. T. Tam and S. T. Seo. 1981. Papaya polygalacturonase and its role in thermally injured ripening fruit. *J. Food Sci.* 46:190.
- Chan, H. T., Jr. and S. Y. T. Tam. The partial separation and characterization of papaya endo-and exo-polygalacturonase. *J. Food Sci.* 47:1478.
- Chan, H. T., Jr. 1986. Effects of heat treatments on the ethylene forming systems in papayas. *J. Food Sci.* 51:581-583.
- Chan, H. T., Jr. 1986. Heat inactivation of the ethylene forming enzyme system in cucumbers. *J. Food Sci.* 51:1491-1493.
- Chan, H. T., Jr. 1988. Delayed light emission as a biochemical indicator of papaya heat treatment. *J. Food Sci.* 53(5):1490-1492.
- Chan, H. T., Jr. 1989. Conditioning cucumbers for quarantine heat treatments. *Hort. Sci.* 24:985.
- Chan, H. T., Jr. 1989. Conditioning cucumbers to increase heat resistance in the EFE system. *J. Food Sci.* 54:1375-1376.
- Chan, H. T., Jr. and J. W. Armstrong. 1990. The effects of various heat treatments on the delayed light emission and ethylene-forming enzyme system in papayas. Symp. "Tropical Fruit in International Trade: Production and Handling." *Int. Soc. Hort. Sci. Honolulu*, June 4-9, 1989. *Acta Horticulture* 269:459-467.

Co-investigators/Cooperators: Need to locate ARS engineer with these special skills.

Work Plans for FY 92: Continue research on relationship between heat sensitivity of EFE enzyme and fruit maturity.

**II.A.1.c.0.2 Armstrong, J. CRIS 5320-43000-009-00D,
02R, 03T**

SY: 1.0

Objective: Develop hot forced air quarantine treatments for California and Hawaii citrus against Mediterranean fruit fly and oriental fruit fly.

Significance: Completion of research in progress and development of quarantine treatments necessary to export citrus from Hawaii to the U.S. mainland or Japan, or from California to other states or Japan in the event of a fruit fly infestation in California. Successful quarantine treatments for citrus exported to Japan and other foreign countries impacts balance of trade.

Justification: Industry - II.G; Regulatory - II.A, II.E.

Constraints: Fruit availability; matching funds from CDFA (02R) and CCRB (03T), the main support for this project, terminate in 1992.

References:

- Armstrong, J. W., J. D. Hansen, B. K. S. Hu and S. A. Brown. 1989. High-temperature quarantine treatment for papayas infested with tephritid fruit flies (Diptera: Tephritidae). *J. Econ. Entomol.* 82:1667-1674.
- Hansen, J. D., J. W. Armstrong, B. K. S. Hu and S. A. Brown. 1990. Thermal death of oriental fruit fly (Diptera: Tephritidae) third instars in developing quarantine treatments for papayas. *J. Econ. Entomol.* 83(1):160-167.
- Armstrong, J. W. 1990. High-temperature forced-air quarantine treatments for fresh fruits infested by tephritid fruit flies. *Acta Horticulture* 269:449-451.
- Gaffney, J. J. and J. W. Armstrong. 1990. High-temperature forced-air research facility for heating fruits for insect oriental fruit fly quarantine treatments. *J. Econ. Entomol.* 83(5):1959-1964.

Patent: Armstrong, J. W., J. D. Hansen, B. K. S. Hu and S. A. Brown. 1990. Hot air disinfestation of fruits and vegetables. U. S. Statutory Invention Registration No. H828, Serial No. 07/270/608. Issued 10/10/90.

Co-investigators/Cooperators: California Department of Food and Agriculture; California Citrus Research Board; Sunkist of California. Potential: ARS, Weslaco, Miami and Fresno.

Work Plans for FY 92: Continue phytotoxicity tests with "Marsh White" and "Marsh Ruby" grapefruit; pummelo cvs.; "Navel", "Valencia", "Ka'u Gold", orange cvs.; and "Bears" lime, including assays for brix, titratable acids, and color and flavor changes. Quarterly progress reports are submitted to CDFA. Complete construction of new forced hot air treatment chamber.

II.A.1.c.0.2 Miller, W. R. CRIS 6617-43000-003-00D

SY: 0.2

Objective: Investigate and determine the limits of physiological tolerance of freshly harvested 'Keitt' mangos to a forced hot-air treatment for quarantine use.

Significance: Florida mango shippers are precluded from shipping to certain Far Eastern markets (since the banning of EDB) due to the lack of a quarantine treatment that will not cause physical or physiological injury. Experiments with the use of irradiation, hot water immersion, and vapor-heat have reportedly caused damage to this fruit. A forced-air treatment at 51.3°C for 125 minutes was determined both entomologically and physiologically successful for 'Tommy Atkins' mangos. Investigations to date with 'Keitt' mango using forced air at 48°C for 180 or 240 minutes or at 51.3°C for 125 minutes resulted in unacceptable ripening and peel damage. However, research will continue to develop a single or double time temperature treatment that will not cause damage to 'Keitt' fruit, yet provide mortality to the pest. The industry will benefit if a single treatment technique such as hot-air can be used on both 'Tommy Atkins' and 'Keitt' fruit. Total economic returns to growers and shippers will increase provided they can market mangos in high-priced export markets. The discovery of nonchemical quarantine treatment for mangos will allow entry of this fruit into markets where U.S. shippers are currently barred.

Justification: Industry - II.G, II.H; Regulatory - II.A, II.E.

Constraints: None.

References:

- Chun, D., W. R. Miller and L. A. Risse. 1987. Effect of curing waxed and individually sealed grapefruit at high and low humidity on storage decay and chilling injury incidence. Hort. Science 22:1055 (abstract).
- Chun, D., W. R. Miller and L. A. Risse. 1987. Effect of high temperature conditioning on waxed and individually film wrapped grapefruit at high and low humidity on storage decay. Proc. 26th Packinghouse Day, PP. 11 (abstract).
- Miller, W. R., D. T. W. Chun, L. A. Risse and T. T. Hatton. 1987. Influence of high temperature conditioning on peel injury and decay of grapefruit after low temperature storage. Proc. Fla. State Hort. Soc. 100:9-12.
- Chun, D., W. R. Miller and L. A. Risse. 1988. Grapefruit storage decay and fruit quality after high-temperature prestorage conditioning at high or low humidity. J. Amer. Soc. Hort. Sci. 113:873-876.
- Miller, W. R., R. E. McDonald, T. T. Hatton and M. Ismail. 1988. Phytotoxicity to grapefruit exposed to hot water immersion treatment. Proc. Fla. State Hort. Soc. 101:192-195.
- Miller, W. R., R. E. McDonald and G. L. Hallman. 1989. Phytotoxicity of hot water and vapor heat treatments to Florida grapefruit. Proc. Int. Inst. Refrig. PP. 207-212.
- Miller, W. R., R. E. McDonald, G. Hallman and J. Sharp. 1991. Condition of Florida grapefruit after exposure to vapor heat quarantine treatment. Hort. Science 26:42-44.
- Miller, W. R., R. E. McDonald and J. Sharp. 1991. Quality changes of 'Tommy Atkins' mangos treated with heated forced air during storage and ripening. Hort. Science 26:395-397.
- Miller, W. R., R. E. McDonald and J. Sharp. 1991. Condition of Florida carambolas after preliminary tests of forced warm air treatment and storage. Proc. Fla. State Hort. Soc. 103:238-241.

Miller, W. R. and R. E. McDonald. 1991. Vapor heat treatment of 'Ruby Red' and 'Marsh' grapefruit. Hort. Science (to editor 10/90, accepted).

Co-investigators/Cooperators: J. R. Brooks and Sons, Homestead, FL; J. Sharp, ARS, Miami.

Work Plans for FY 92: Previous work during FY 91 at the U. S. Horticultural Research Laboratory, Orlando, FL, shows that mangos of cv 'Keitt' will not tolerate physiologically (uneven ripening of pulp tissue) the stress of hot air treatments at 51.3C for 125 minutes (this treatment is satisfactory for cv 'Tommy Atkins'), or 48C for 180 or 240 minutes. Will investigate the potential of a two temperature stage forced hot-air treatment such as lower temperature followed by a higher temperature or a higher temperature followed by a lower temperature (specific time and temperatures not yet determined) until center pulp temperature of 43.5C is maintained for about 30 minutes. Hot-air treatment will be explored first and if not successful will be followed by experiments using vapor heat.

II.A.1.c.0.2 Mitcham, E. J. CRIS 6617-430000-003-00D

SY: 0.4

Objective: Determine the biochemical basis for damage, including textural disorders, which occurs in mango fruit after high temperature quarantine treatments.

Significance: Mango fruit which are heat-treated for quarantine purposes are often significantly damaged (hard spots in flesh). Such damage reduces the marketability of the crop. Less damaging treatments are greatly needed. An understanding of the biochemical basis for high temperature injury of fruits will allow an informed approach towards developing effective quarantine treatments which will not result in loss of fruit quality. Also, identification of a biochemical marker of high temperature injury would allow for rapid determination of the occurrence of injury and its severity. This information would greatly aid treatment development.

Justification: Industry - II.C, II.E, II.G, II.H; Regulatory - II.A, II.E. Although none of the needs specifically calls for research leading to an understanding of the basis of injury resulting from high temperature quarantine treatments, several call for development of alternative, improved or new quarantine treatments. These needs indicate dissatisfaction with present quarantine treatments for some crops and the need

for the development of an approved quarantine treatment for several crops. An understanding of the biochemical basis for the injury which sometimes develops after high temperature exposure will allow an informed approach towards developing new and improved treatments. If the fruit quality is severely damaged by the quarantine treatment, the benefit of shipping that product is greatly reduced if not eliminated.

Constraints: Before the effects of high temperatures on mango fruit texture can be studied, a basic understanding must be gained of ripening-associated textural changes. Efforts are currently focused in this area.

References:

Mitcham, E. J. and R. E. McDonald. Effect of high temperature on cell wall modifications associated with tomato (*Lycopersicon esculentum* Mill.) fruit ripening. Postharvest Biology and Technology (submitted).

Co-investigators/Cooperators: R. E. McDonald and W. R. Miller, ARS, Orlando; J. R. Brooks and Sons, Inc., Miami.
Potential: The Florida Mango Forum, Inc.

Work Plans for FY 92: As textural changes in fleshy fruits are largely determined by cell wall structure, we will determine the effects of a range of high temperature treatments on mango cell wall structure and the activity of several ripening-related enzymes including polygalacturonase and cellulase. We will begin to determine the limit of mango fruit tolerance to high temperature exposure. Fruit respiration during and following high temperature exposure will also be investigated to determine if an oxygen deficit develops in the center of the fruit during heat treatment.

II.A.1.c.0.2 Mitcham, E. J. CRIS 6617-430000-003-00D

SY: 0.3

Objective: Explore the potential benefit of temperature conditioning of fruits prior to quarantine heat treatment.

Significance: Physiological data may show that certain fruits will not withstand the high temperatures required for fly mortality without sustaining injury. Under these circumstances, use of a conditioning treatment may allow the fruit to undergo the necessary quarantine heat treatment without injury.

Justification: Industry - II.C, II.G, II.H; Regulatory - II.A, II.E. Several needs call for development of improved quarantine treatments. High temperature treatments which have previously caused fruit injury may be modified with a temperature conditioning pretreatment to reduce or eliminate injury to the fruit while providing quarantine security.

Constraints: Both the conditioning treatment and the quarantine treatment must be commercially useful in a consistent, reliable manner. Fly mortality must be confirmed over the entire treatment regime to ensure that flies are not also conditioned to better withstand the high temperature exposure.

References: None.

Co-investigators/Cooperators: R. E. McDonald and W. R. Miller, ARS, Orlando; J. Sharp, ARS, Miami; J. R. Brooks and Sons, Inc., Miami. Potential: ARS, Miami; The Florida Mango Forum, Inc.

Work Plans for FY 92: Explore the benefit of exposure of mango fruit to high, yet non-damaging temperatures prior to exposure to temperatures and durations required for quarantine heat treatments. Monitor high-temperature injury through electrolyte leakage and activity of ripening-associated enzymes.

II.A.1.c.0.2 Miller, W. R. CRIS 6617-43000-003-00D

Also: II.A.1.d

SY: 0.05

Objective: Initiate investigation of the physiological response and physical stress tolerances of some high value tropical fruits such as lychee and passion fruit when exposed to treatments of hot-air, vapor-heat, or cold temperatures as potential quarantine treatments against the Caribbean fruit fly.

Significance: The production of lychee and passion fruit is expanding in south Florida. There are many relatively small growers providing fruit to very few packers/shippers; the total volume available to shippers for marketing is rapidly increasing. The availability of an effective quarantine treatment that will not damage fruit and allow normal physiological changes after treatment and storage will eliminate the only barrier to export to many Pacific rim countries. Export sales will increase revenue to both growers and shippers.

Justification: Industry - II.G, II.H; Regulatory - II.A, II.E.

Constraints: None.

References:

- Miller, W. R, D. Chun, L. A. Risse, T. T. Hatton and T. Hinsch. 1990. Conditioning of Florida grapefruit to reduce peel stress during low-temperature storage. Hort. Science 25:209-211.
- Miller, W. R. and R. E. McDonald. 1990. Alternative quarantine treatments for citrus. Proc. of 29th Annual Citrus Packinghouse Day Seminar. PP. 12 (abstract).
- Miller, W. R, R. E. McDonald and J. Sharp. 1991. Quality changes of 'Tommy Atkins' mangos treated with heated forced air during storage and ripening. Hort. Science 26:395-397.

Co-investigators/Cooperators: J. Sharp, W. Gould, and G. Hallman, ARS, Miami; Florida growers; Florida shippers.

Work Plans for FY 92: In preliminary investigations, lychees (Litchi chinensis) will be subjected to various temperatures and durations of time with heated air and/or vapor heat and the response of fruit after subsequent storage will be evaluated and documented.

d. Cold

II.A.1.d Miller, W. R. CRIS 6617-43000-003-00D

SY: 0.3

Objective: Investigate and develop procedures for the successful application of cold treatment for quarantine purposes to carambola fruit that will not cause damage to fruit or identify another alternative nonchemical treatment such as forced hot air, vapor heat or hot water immersion.

Significance: A cold treatment (1°C for 15 days) is presently in use for domestic shipments only. However, the treatment causes serious damage to fruit and shippers state that marketing losses are excessive. If the cold treatment can be consistently applied to carambolas without fruit injury, approval can be obtained for export to Far Eastern markets. The shippers in south Florida have placed a high priority on the successful development of a viable quarantine treatment for carambolas. Volume of production is rapidly increasing and shippers expect sufficient supplies of this high-value commodity to be available for export. Thailand and Malaysia have recently entered the Japanese carambola market and U.S. shippers are very interested in following. Since carambolas are produced for about 10 months of the year,

significant increased revenue for growers and shippers is expected. Opening new export markets will assure that increased supplies will not depress the relatively high domestic prices that growers and shippers now enjoy.

Justification: Industry - II.G, II.H; Regulatory - II.A, II.E.

Constraints: None.

References:

- Miller, W. R., D. Chun, L. A. Risse, T. T. Hatton and T. Hinsch. 1990. Conditioning of Florida grapefruit to reduce peel stress during low-temperature storage. Hort. Science 25:209-211.
- Miller, W. R. and R. E. McDonald. 1990. Vapor heat treatment of Florida grapefruit for the Caribbean fruit fly. Proc. XXIII Int. Hort. Cong. Vol. 2:3347. Florence, Italy (abstract).
- Miller, W. R. and R. E. McDonald. 1990. Alternative quarantine treatments for citrus. Proc. of 29th Annual Citrus Packinghouse Day Seminar. PP. 12 (abstract).
- Miller, W. R., R. E. McDonald and J. L. Sharp. 1991. Condition of Florida carambolas after preliminary tests of forced warm air treatment and storage. Proc. Fla. State Hort. Soc. 103:238-241.

Co-investigators/Cooperators: J. Sharp and W. Gould, ARS, Miami; Florida growers; Florida shippers.

Work Plans for FY 92: Continue investigations on the refinement of currently used cold treatment procedures (1C for 15 days) for the purpose of reducing rapid physiological deterioration of fruit during storage after exposure to commercial cold treatment. Experiments of cold treatment will be conducted with and without prior preconditioning at 15C for 3 days, with or without applying tissue wraps (commercially used) and with or without fruit coatings.

II.A.1.d Gould, W.

CRIS 6631-43000-005-00D

SY: 0.3

Objective: Determine the cold tolerance of larvae and eggs both in various varieties of fruit and exposed stages, and correlate mortality with the supercooling point. Determine the effect of raising temperature for various periods of time. Determine if the insects can be acclimated to cold or heat.

Significance: Does the larval substrate have an effect on a quarantine treatment or can results from one commodity be extrapolated to other commodities. Can the supercooling point be used to bypass more time consuming determinations of cold mortality. If acclimation to environmental conditions exists, it could affect

quarantine treatments adversely and would have to be compensated for in initial setup of the treatments. Will be able to determine if a refrigeration breakdown during a quarantine treatment will invalidate a given treatment.

Justification: Industry - II.D; Regulatory - II.A, II.E, III.CC.

Constraints: A limited number (2) of incubators is available to work at different temperatures. Additional cold units with precision for research would cost \$5-7000 each.

References:

- Gould and Sharp. 1990. Cold storage quarantine treatment for carambolas infested with the Caribbean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 83(2):458-460.
- Gould. 1988. A hot water/cold storage quarantine treatment for grapefruit infested with the Caribbean fruit fly. Proc. Fla. State Hort. Soc. 101:190-192.

Co-investigators/Cooperators: J. Sharp, ARS, Miami.

Work Plans for FY 92: The cold experimental work will continue beyond FY 92.

II.A.1.d Yokoyama, V. Y. CRIS 5302-43000-016-00D

SY: 0.2

Objective: Develop a quarantine treatment using low temperature storage to replace methyl bromide fumigation to control walnut husk fly, Rhagoletis completa, in stone fruits for export to New Zealand.

Significance: New Zealand suspended shipments of stone fruits from California in 1989 due to potential walnut husk fly infestations. A methyl bromide fumigation is under development as a quarantine treatment to control walnut husk fly in peaches in order to eliminate the ban. Low temperature storage will be investigated as a long-term solution to replace methyl bromide to control walnut husk fly in stone fruits. The ban on stone fruits due to potential walnut husk fly infestations jeopardizes a \$43 million market in Pacific Rim countries. Low temperature storage for control of walnut husk fly can be economically incorporated into normal postharvest handling techniques for stone fruits.

Justification: Industry - II.G, II.H; Regulatory - II.E.

Constraints: None.

References:

- Yokoyama, V. Y., G. T. Miller and P. L. Hartsell. 1991. Pest-free period and methyl bromide fumigation for control of walnut husk fly (Diptera: Tephritidae) in stone fruits exported to New Zealand. J. Econ. Entomol. 84: (In press).
- Yokoyama, V. Y. and G. T. Miller. 1989. Response to codling moth and oriental fruit moth (Lepidoptera: Tortricidae) immatures to low-temperature storage of stone fruits. J. Econ. Entomol. 82:1152-1156.

Co-investigators/Cooperators: California Tree Fruit Agreement; California Department of Food and Agriculture; California grower-shippers.

Work Plans for FY 92: Evaluate the effect of low temperature storage on the survival of immature stages of walnut husk fly.

II.A.1.d Gould, W.

CRIS 6631-43000-005-00D

SY: 0.1

Objective: Cold torpor: At what temperature do the flies cease activity and egg laying. What is the output of eggs at various temperatures.

Significance: For quarantine treatments it is important to know the extent of the pest population during different times of the year. Cold has an important effect on flight activity and fecundity which affect the extent of infestations in a commodity.

Justification: Regulatory - II.B, III.X, III.CC, III.PP.

Constraints: A limited number (2) of incubators is available to work at different temperatures. Additional cold units with precision for research would cost \$5-7000 each.

References: None.

Co-investigators/Cooperators: J. Sharp, ARS, Miami.

Work Plans for FY 92: Initiate study as incubator space permits. The work will continue beyond FY 92.

Also see Miller II.A.1.c.0.2.

e. Electromagnetic

II.A.1.e Armstrong, J.

CRIS 5320-43000-009-00D

Also: II.C

SY: 1.5

Objective: Research and develop alternatives to the presently available quarantine treatment technologies including: 1) Further research into controlled or modified atmospheres at low temperatures for short durations; 2) Review of microwave technologies to determine whether new advances in microwave physics will allow the specific targeting of fruit fly eggs and larvae within infested fruits; 3) Investigation of effects of electromagnetic fields, directed radio frequency waves, high voltage-low amperage and low voltage-high amperage electrical currents, and other electrical energy effects on fruit fly eggs and larvae in vitro and in vivo; 4) Determine the feasibility of flash-freezing for certain fruit fly hosts of economic importance and 5) Determine the feasibility of new technologies for non-invasive detection of fruit fly eggs and larvae, and other important quarantine pests, in infested fruits for use in automated culling systems.

Significance: There are no available quarantine treatments against fruit flies for many fruits of economic importance. Alternative quarantine treatments must be found for those fruits that cannot tolerate heat or cold at temperatures and times required to kill fruit fly eggs and larvae. Alternatives to methyl bromide fumigation must be found because the continued registration of this toxic fumigant is questionable. Successful quarantine treatments for fruits exported to Japan and other foreign countries impacts balance of trade.

Justification: Industry - II.A, II.G, II.H; Regulatory - II.A., II.E.

Constraints: Availability of equipment and expert consultation in new areas of energy research which are difficult to locate and generally found in the defense industry thereby limiting accessibility.

References: None.

Co-investigators/Cooperators: Potential: University of Hawaii; Pasadena Jet Propulsion Laboratory; Los Alamos and Oak Ridge Research Centers; Federal, state (university) and private research institutions working with high-energy/plasma physics; ARS, Weslaco, Miami and Fresno.

Work Plans for FY 92: Initiate contacts in the area of high-energy/plasma physics; determine availability of

equipment and safety requirements involved with research into this area. Determine feasibility of different technologies as potential quarantine treatments.

f. Irradiation

2. Systems Approach

Background - An integrated approach to quarantine security involves calculating the probability of commodity infestations based on all treatments, both pre- and post-harvest. These can be multiple post-harvest treatments and detection programs integrated with field treatments that reduce populations and the probability that commodities will be infested. The approach combines knowledge of the basic biology of the pest and host with population, community and systems ecology to devise production, harvest and post-harvest procedures to attain quarantine security.

Needs - Information concerning the basic biology of the pest and host is required to determine optimal strategies. Specific knowledge is lacking for many commodities and tephritid species including host status relative to fruit species, genetic and varietal differences, and maturation stages. Pest population models are necessary for determining possible pest-free periods and integrated pesticide treatments. Behavioral and physiological information concerning modes of infestation, methods for detection and possible elimination of specific fruit fly populations are needed.

*II.A.2 Hennessey, M. K. CRIS 6631-43000-007-00D
Sharp, J. L.*

SY: 1.75

Objective: Develop and implement systems approach in establishing quarantine security for various commodities against Caribbean fruit fly and papaya fruit fly.

Significance: When implemented, the systems approach will justify elimination of some post-harvest treatments such as fumigation and irradiation for some commodities. Lowering levels of fumigant residues in foods and reducing use of gamma irradiation is desirable in today's consumer markets.

Justification: Industry - II.B, II.E, II.G, II.H;
Regulatory - II.A, II.B, II.D, II.F.

Constraints: Time to evaluate some commodities over several growing season in the field. Availability of colony-reared flies for research. Availability of adequate numbers of commercially grown and harvested commodities and commercial groves for research purposes.

References: None.

Co-investigators/Cooperators: Florida Dept. of Plant Industry; Florida subtropical fruit processors.

Potential: University of Florida.

Work Plans for FY 92: Determine, through field experiments, periods when commodities are non-hosts, resistant, or flies are not present.

a. Host Resistance

0.1 Biochemical resistance

II.A.2.a.0.1 Shapiro, J. P. CRIS 6617-22000-007-00D

Also: II.A.2.a.0.2

SY: 0.3

Objective: Investigate the use of natural products such as flavonoids to reduce the incidence of Caribbean fruit fly attacks on citrus.

Significance: Certain flavonoids such as eriodictyol have been shown to have antibiotic effects on insect growth and development. Flavonoids are present in high amounts in citrus fruits and vary from one citrus variety to another. It is possible to control the levels of flavonoids by cloning the genes for the enzymes responsible for the synthesis of inhibitory flavonoids and inserting them into desired citrus varieties. Once the genes are cloned for the flavonoid enzymes they could be inserted into appropriate plants. Metabolism of flavonoids by insects would also be investigated.

Justification: Industry - II.B, II.G; Regulatory - II.D, II.F, III.EEE.

Constraints: The enzyme systems responsible for the synthesis of the desired flavonoids must be characterized, antibodies to the enzymes prepared and the genes cloned. Cell cultures of the varieties must be available for gene insertion. Analyses of the enzymes in the transformed plants must be performed. These tasks may be limited by lack of manpower.

References: None.

Co-investigators/Cooperators: A. Waiss, ARS, Albany; P. Greany, ARS, Gainesville; R. Niedz, W. Schroeder, and R. Mayer, ARS, Orlando. Potential: M. Burke, Univ. of Aberdeen, Aberdeen, Scotland; W. Heller, GSF, Munich, Germany.

Work Plans for FY 92: Oranges, grapefruits, lemons and limes will be harvested from early stages of development through maturity. Simultaneously, several cultures of embryogenic and nonembryogenic callus will be grown. An expedient assay for flavonone 3'-hydroxylase is being developed and will be used to test microsomes isolated from both fruit peel and callus tissue. Microsomes will be probed with antibodies to mammalian hydroxylases to test for cross reactivity. The ability of fruits to synthesize eriodictyol from naringenin will be correlated with resistance to Caribbean fruit fly larva. With discovery of a good source of enzyme in either fruit or callus, enzyme characterization and isolation will begin, with the goal of deriving specific amino acid sequences and identifying gene sequences coding for the enzyme(s).

II.A.2.a.0.1 Waiss, A.

CRIS 3325-21220-012-00D

SY: 0.2

Objective: Establish the chemical basis of fruit fly resistance in citrus as related to flavonoid content.

Significance: Certain cultivars of lemon and lime have demonstrated to be resistant to fruit fly attack. From published data, there is a strong correlation between their content of the glycosides of eriodictyol and the observed resistance. This is consistent with earlier studies showing that eriodictyol and other catecholic flavonoids exhibit suppressive effects upon the growth and development of several insects. In establishing a chemical basis for citrus resistance to fruit flies we will be able to proceed rationally in improving this resistance to this pest either by classical plant breeding or through modern methods of genetic alteration. Such resistance will have significant economic benefits inasmuch as interstate shipment and foreign export of citrus fruit will be possible without costly treatment to eliminate fruit fly infestation.

Justification: Industry - II.H; Regulatory II.E.

Constraints: Gram quantities of citrus flavonoids must be isolated and purified in order to study the inhibitory

activities of these compounds upon fruit flies. A minimal diet for both Mediterranean and Caribbean fruit flies must be established and used so that a relevant correlation can be made between the chemical structures of the citrus flavonoids and possible synergistic actions of citrus monoterpenes on the growth and development of these insects.

References:

Elliger, C. A., B. G. Chan and A. C. Waiss, Jr. 1980. Flavonoids as larval growth inhibitors: Structure factor governing toxicity. *Naturwissen* 67:358-359.

Co-investigators/Cooperators: P. Greany, ARS, Gainesville; E. Jang, ARS, Hilo; T. Williams, ARS, Riverside.

Work Plans for FY 92: 1) Citrus fruits at two stages of maturity will be selected from the citrus repository in Riverside, CA (Williams) and sent to Hawaii (Jang) for medfly bioassay and Albany, CA (Elliger) for chemical analysis. Correlations will be made between flavonoid contents in citrus albedo and medfly survival and growth. 2) Gram quantities of citrus flavonoids will be isolated and purified to facilitate the investigation on the growth inhibitory activities of these compounds upon fruit flies.

II.A.2.a.0.1 Waiss, A. CRIS 3325-21220-012-00D

SY: 0.2 (Waiss), 1.0 (Bragg)

Objective: Transgenic introduction of fruit fly resistance gene into susceptible citrus cultivars.

Significance: Certain ortho-dihydroxy substituted flavonoids have been shown to retard or inhibitory insect growth and development in numerous plants. Recent experiments indicate that fruit fly resistance in certain varieties of lemon and lime correlate directly to this type of flavonoid content within the citrus fruit albedo. The transfer of fruit fly resistance into formerly susceptible citrus may be accomplished through the insertion of an ortho-hydroxylase gene (grapefruit) or by manipulation of a methyl transferase gene (orange). These genetic techniques should be applicable to other crops as well.

Justification: Industry - II.H; Regulatory - II.E.

Constraints: The enzymes responsible for oxidation of naringin in grapefruit and for methyl transfer in orange must be isolated and purified. They must be sequenced in order for the respective genes to be identified and cloned. It is intended that antisense DNA corresponding to the methyl transferase gene be cloned. Appropriate cell cultures must be obtained and technology for gene insertion in citrus be established.

References:

Waiss, Jr., A. C., B. G. Chan, C. A. Elliger, D. L. Dryer, R. G. Binder and R. C. Guedner. 1981. Insect growth inhibitors in crop plants. Proc. 1st Japan-USA Sym. on Integrated Pest Management, Tsukuba, Japan, PP 75-91.

Co-investigators/Cooperators: R. Mayer and R. Niedz, ARS, Orlando; D. Ow, ARS, Albany.

Work Plans for FY 92: Methyl transferase from orange will be isolated and purified.

II.A.2.a.0.1 Greany, P. D. CRIS 6615-22000-003-00D

SY: 0.1

Objective: Complete elucidation of immunity of lemons to tephritids.

Significance: The differential susceptibility of lemons, oranges and grapefruit correlates well with corresponding differences in flavonoid content, both qualitatively and quantitatively. We are now investigating whether known differences in the flavonoid composition also have a causal relationship to fruit fly susceptibility differences among these cultivars, and whether flavonoids synergize the action of deleterious terpenoids in the peel oil. By defining the mechanism(s) affording immunity of lemons to fruit flies, it may be possible to enhance the resistance of grapefruit and oranges through incorporation or manipulation of resistance genes. Success in this effort could confer non-host status for all citrus cultivars, precluding the need for any other treatments.

Justification: Regulatory - II.D, II.F.

Constraints: None anticipated.

References:

Greany, P. D., Styer, S. C., Davis, P. L., Shaw, P. E., and Chambers, D. L. 1983. Biochemical resistance of citrus to fruit flies: Demonstration

- and elucidation of resistance to the Caribbean fruit fly, Anastrepha suspensa. Ent. exp. & appl. 34: 40-50.
- Styer, S. C., and Greany, P. D. 1983. Increased susceptibility of laboratory-reared vs. wild Caribbean fruit fly, Anastrepha suspensa (Loew) (Diptera: Tephritidae), larvae to toxic citrus allelochemicals. Environ. Entomol. 12: 1606-08.
- Greany, P. D., Shaw, P. E., Davis, P. L., and Hatton, T. T. 1985. Senescence-related susceptibility of Marsh grapefruit to laboratory infestation by Anastrepha suspensa (Diptera: Tephritidae). The Fla. Entomol. 68:144-150.
- McDonald, R. E., Shaw, P. E., Greany, P. D., Hatton, T. T., and Wilson, C. W. 1987. Effect of gibberellic acid on certain physical and chemical properties of grapefruit. Trop. Sci. 27: 17-22.
- Greany, P. D., McDonald, R. E., Shaw, P. E., Schroeder, W. J., Howard, D. F., Hatton, T. T., Davis, P. L., and Rasmussen, G. K. 1987. Use of gibberellic acid to reduce grapefruit susceptibility to attack by the Caribbean fruit fly, Anastrepha suspensa (Diptera: Tephritidae). Trop. Sci. 27: 261-270.
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- Wilson, C. W., III, Shaw, P. E., McDonald, R. E., Greany, P. D., and Yokoyama, H. 1990. Effect of gibberellic acid and 2-(3,4-dichlorophenoxy) triethylamine on nootkatone in grapefruit peel oil and total peel oil content. J. Ag. Food Chem. 656-659.
- Rössler, Y., and Greany, P. D. 1990. Enhancement of citrus resistance to the Mediterranean fruit fly. Entomol. exp. & appl. 54: 89-96.
- Shaw, P. E., Calkins, C. O., McDonald, R. E., Greany, P. D., Webb, J. C., Nisperos-Carriedo, M. O., and Barros, S. M. 1991. Changes in limonin and naringin levels in grapefruit albedo with maturity and the effects of gibberellic acid on these changes. Phytochem. (accepted April, 1991).
- Greany, P. D., McDonald, R. E., Schroeder, W. J., and Shaw, P. E. 1991. Improvement in the efficacy of gibberellic acid treatments in reducing susceptibility of grapefruit to attack by the Caribbean fruit fly, Anastrepha suspensa. The Florida Entomologist (in press).

Co-investigators/Cooperators: P. Shaw, ARS, Winter Haven; J. Shapiro, R. Mayer, R. McDonald, Orlando; T. Waiss, ARS, Albany.

Work Plans for FY 92: To develop microassay allowing dose-response and structure-activity data to be obtained for flavonoids from grapefruit, oranges and lemons and to initiate tests on possible synergistic effects of terpenoids (from peel oil) and flavonoids.

II.A.2.a.0.1 Mayer, R. T. CRIS 6617-22000-007-00D

Also: II.A.2.a.0.2

SY: 0.2

Objective: Investigate chitinases as a possible control strategy for controlling the Caribbean fruit fly.

Significance: Chitin is a major component of the insect exoskeleton and the peritrophic membrane that lines the insect intestinal tract. Chitinases are present in insects and function as a defensive mechanism against fungal invasion as well as being heavily involved in the insect molting process. Disruption of chitin-containing structures would make insects more vulnerable to infection by pathogens while interference with the insect chitinases at the time of the molt would disrupt the molting process. Although plants do not contain chitin or chitosan, they do possess chitinases and chitosanases. These enzymes can be induced by stress, ethylene, and pathogens, and are considered to be pathogenesis-related proteins. Enhancement or induction of chitinases and chitosanases may aid in controlling insect herbivores by making them more susceptible to insect pathogen attack. Inhibitors or metabolic disruptors of fruitfly chitinases would provide a new control strategy.

Justification: Industry - II.B, II.G; Regulatory - II.D, II.F, III.EEE.

Constraints: Citrus and Caribbean fruit fly chitinases must be characterized, antibodies to the enzymes prepared, and the genes cloned. Cell cultures of the varieties must be available for gene insertion. Analyses of the enzymes in the transformed plants must be performed. Manpower to perform experiments will be necessary.

References: None.

Co-investigators/Cooperators: R. Niedz, W. Schroeder, J. Shapiro and R. McDonald, ARS, Orlando; W. Osswald, Technische Universitat, Munich, Germany. **Potential:** P. Greany, ARS, Gainesville.

Work Plans for FY 92: Chitinase levels in four varieties of citrus will be ascertained and tests on susceptibility to Caribbean fruit flies will be made through the growing season. Chitinase levels will also be determined for the Caribbean fruit fly and the enzyme will be characterized in regard to activity.

0.2 Physiology

II.A.2.a.0.2 Yokoyama, V. Y. CRIS 5302-43000-016-00D

SY: 0.2

Objective: Evaluate the status of peaches, nectarines and plums as hosts for walnut husk fly, Rhagoletis completa.

Significance: Shipments of stone fruits from California have been suspended by New Zealand due to potential walnut husk fly infestations. Peaches are a documented host for walnut husk fly but the status of nectarines and plums as hosts is unknown. Laboratory and field tests will be conducted to determine the acceptability of each stone fruit species for oviposition and complete development of immature stages in the fruit. The establishment of the non-host status for any stone fruit species will eliminate the need for quarantine treatments and enhance the export market by shipment of economical fruit of high quality.

Justification: Industry - II.H; Regulatory - II.D.

Constraints: None.

References:

- Yokoyama, V. Y., G. T. Miller and P. L. Hartsell. 1990. Evaluation of a methyl bromide quarantine treatment to control codling moth (Lepidoptera: Tortricidae) on nectarine cultivars proposed for export to Japan. J. Econ. Entomol. 83:466-471.
- Yokoyama, V. Y. and G. T. Miller. 1988. Laboratory evaluations of codling moth (Lepidoptera: Tortricidae) oviposition on three species of stone fruit grown in California. J. Econ. Entomol. 81:868-872.
- Yokoyama, V. Y. and G. T. Miller. 1988. Laboratory evaluations of oriental fruit moth (Lepidoptera: Tortricidae) oviposition and larval survival on five species of stone fruits. J. Econ. Entomol. 81:867-872.

Co-investigators/Cooperators: California grower-shippers; California Department of Food and Agriculture; California Tree Fruit Agreement.

Work Plans for FY 92: Evaluate three species of stone fruits as hosts for walnut husk fly in laboratory and field tests.

II.A.2.a.0.2 Greany, P. D. CRIS 6615-22000-003-000

SY: 0.25

Objective: To evaluate under actual field conditions the use of gibberellic acid (GA) treatments for prophylaxis against damage by Anastrepha spp. and Ceratitidis capitata.

Significance: 1) By using GA to extend innate resistance of citrus fruit to tephritids, it may be possible to eliminate or delay use of pesticides and therefore to avoid yield loss in areas inhabited by the most damaging

tephritids. 2) In areas where fruit flies pose primarily a quarantine threat, GA may be useful in extending the duration of "fly-free" windows and in extending fly free geographic areas. This agent already is EPA-approved for use on citrus. It does not affect internal fruit ripening. Because it is non-toxic, it should not adversely affect biological control agents important in controlling other citrus pests; i.e., it is a biorational control agent.

Justification: Industry - II.G, II.H.

Constraints: Demonstration of efficacy in the field will require either a damaging population of wild flies, or use of field cages within which GA-treated vs. untreated fruit will be exposed to the tephritid species of concern. Availability of wild flies for use in field cage tests can be a problem due to the phenology of citrus fruit, which generally ripen during the late fall and winter months.

References: See II.A.2.a.0.1 Greany.

Co-investigators/Cooperators: R. McDonald and W. Schroeder, ARS, Orlando; P. Shaw, ARS, Winter Haven; C. Calkins, ARS, Gainesville; A. Malavasi, Univ. of Sao Paulo, Brazil; M. Aluja, Inst. de Ecologia, Veracruz, Mexico; Y. Rössler, Israel. Potential: APHIS S&T; S. Simpson, C. Rihard, Florida Dept. of Agriculture.

Work Plans for FY 92: To perform field cage tests to determine the optimal formulation of GA and surfactant for cost-benefit purposes, toward initiating a full-scale pilot test in FY 93.

II.A.2.a.0.2 Greany, P. D. CRIS 6615-22000-003-00D

SY: 0.05

Objective: Determine validity of peel senescence criteria (peel color, puncture resistance, and selected chemical properties) for use in defining fruit susceptibility to fruit flies.

Significance: We believe one or more of these indices can be used to judge the potential susceptibility of fruit to fruit fly attack, thereby allowing increased security for shipment of fruit to quarantine-sensitive areas under "fly free" protocols.

Justification: Industry - II.G, II.H.

Constraints: None, other than availability of funding to carry out tests.

References: See II.A.2.1.0.1 Greany.

Co-investigators/Cooperators: R. McDonald and W. Schroeder, ARS, Orlando; P. Shaw, ARS, Winter Haven; C. Calkins and V. Chew, ARS, Gainesville; A. Malavasi, Univ. of Sao Paulo, Brazil; M. Aluja, Inst. de Ecologia, Veracruz, Mexico; Y. Rössler, Israel. Potential: APHIS, S&T; S. Simpson, C. Riherd, DPI, Florida Dept. of Agriculture.

Work Plans for FY 92: To develop statistical methods to correlate fruit color, puncture resistance and peel oil content with fly susceptibility.

II.A.2.a.0.2 McDonald, R. CRIS 6617-43000-003-00D

SY: 0.1

Objective: Determine if 'Marsh' and 'Ruby Red' grapefruit treated preharvest with gibberellic acid (GA) will mitigate the damage of a vapor-heat treatment (43.5°C for about 4 hours) as a potential quarantine treatment.

Significance: Early season Florida grapefruit exported to quarantine markets comes from the fly free zone and the approved cold treatment is recommended and used by the industry on fruit harvested during the latter half of the production season. Alternative quarantine treatments for grapefruit are needed by the industry. Application of GA to grapefruit trees before fruit color break will delay normal physiological senescence of peel tissue. The delay of peel maturity will lessen the damaging effects of the potential vapor-heat quarantine treatment. This will allow export of high quality fruit late in the season when typical fruit quality is on the decline and improve the general competitiveness of U.S. shippers relative to other sources of supply in the same export markets. The cold treatment requires about three weeks to complete whereas a vapor-heat treatment can be conducted in about 4.5 hours.

Justification: Industry - II.G, II.H; Regulatory - II.A, II.E.

Constraints: None.

References:

- McDonald, R. E, P. E. Shaw, P. D. Greany, T. T. Hatton, and C. W. Wilson. 1987. Effect of gibberellic acid on certain physical and chemical properties of grapefruit. *Trop. Sci.* 27:17-22.
- McDonald, R. E, P. D. Greany, P. E. Shaw, W. J. Schroeder, T. T. Hatton and C. W. Wilson. 1988. Use of gibberellic acid for Caribbean fruit fly (Anastrepha suspensa) control in grapefruit. *Proc. Sixth Intl. Citrus Cong.* 3:1147-1152.

Co-investigators/Cooperators: W. Miller, ARS, Orlando.
Potential: J. Sharp, ARS, Miami.

Work Plans for FY 92: 'Marsh' grapefruit tress will be sprayed with 10 ppm gibberellic acid prior to color break (September). Triplicate harvests will be made in February of treated and control fruit which will be subjected to a vapor-heat treatment (43.5C for 4 hours). Fruit quality will be determined after holding in storage to simulate an export shipment to determine if the gibberellic acid will mitigate the injurious effects of the vapor-heat treatment.

II.A.2.a.0.2 McDonald, R. CRIS 6617-43000-003-00D

SY: 0.3

Objective: Continue studies to establish easily measured properties of grapefruit related to Caribbean fruit fly susceptibility.

Significance: Based on inherent early season resistance, grapefruit can be shipped to export markets until December 20 if it is produced in the fly-free zone. The December 20 date is an arbitrary date and it is not based on any physical property of the fruit. It would be useful if we were able to relate the easily measured properties of peel puncture resistance and color to fruit fly susceptibility. This would allow the establishment of a susceptibility threshold based on quantitative measurements of fruit condition, rather than date of harvest. Research has shown that peel maturity can be delayed by a preharvest application of gibberellic acid (GA). GA treatments may allow an extension of the "window of resistance" based upon the physical properties of the fruit. This would be a distinct advantage to growers/shippers because no postharvest treatment would be required to meet quarantine restrictions.

Justification: Industry - II.G, II.H; Regulatory - II.A, II.E.

Constraints: To test this methodology, we need to purchase testing equipment that would be suitable for tests on peel color and puncture resistance, and be

computer compatible so that the data could easily be analyzed.

References:

- McDonald, R. E., P. E. Shaw, P. D. Greany, T. T. Hatton and C. W. Wilson. 1987. Effect of gibberellic acid on certain physical and chemical properties of grapefruit. *Trop. Sci.* 27:17-22.
- Greany, P. D., R. E. McDonald, P. E. Shaw, W. J. Schroeder, D. F. Howard, T. T. Hatton, P. L. Davis and G. K. Rasmussen. 1987. Use of gibberellic acid to reduce grapefruit susceptibility to attack by the Caribbean fruit fly Anastrepha suspensa (Diptera: Tephritidae). *Trop. Sci.* 27:261-270.
- McDonald, R. E., P. D. Greany, P. E. Shaw, W. J. Schroeder, T. T. Hatton and C. W. Wilson. 1988. Use of gibberellic acid for Caribbean fruit fly (Anastrepha suspensa) control in grapefruit. *Proc. Sixth Intl. Citrus Cong.* 3:1147-1152.

Co-investigators/Cooperators: P. Greany, ARS, Gainesville. Potential: S. Simpson, DPI, Florida State Department of Agriculture, Winter Haven.

Work Plans for FY 92: Gibberellic acid (GA) treatments will be applied prior to color break by dipping 50 individual "Marsh" grapefruit with 10 ppm GA and 0.1% L-77 surfactant and 50 fruit with 10 ppm GA and 0.05% L-77 surfactant. There will also be 50 control fruit on each tree. At seven monthly intervals, eight fruit from each treatment from each of three replicate trees will be harvested and peel oil and limonin content will be determined. At the same time, field cages will be placed over the three replicate trees and Caribbean fruit flies will be released. After three days, 33 fruit from each treatment and control fruit will be harvested and peel oil and puncture resistance will be determined on one-half of each treatment. All fly-exposed fruit will then be held in individual containers to determine fruit fly puparia and adult development. Correlations will be made between the physical properties of individual fruit and susceptibility to attack by fruit flies.

Also see Shapiro II.A.2.a.0.1, Mayer II.A.2.a.0.1.

b. Population Reduction

0.1 Bait

0.2 Sanitation

0.3 Male annihilation

0.4 Mass trapping

c. Host Status

II.A.2.c Hennessey, M. K. CRIS 6631-43000-007-00D

Sharp, J. L.

SY: 1.75

Objective: Demonstrate non-host status of some current Caribbean fruit fly hosts.

Significance: Non-host status will open new markets, influence establishment of new varieties and eliminate need for post-harvest treatments. Such status will also contribute to overall strategy of systems approach.

Justification: Industry - II.B, II.E, II.H; Regulatory - II.A, II.B, II.D, II.F.

Constraints: Time to evaluate many varieties over several growing seasons. Availability of colony flies, harvested commodities and grove space for experiments.

References: None.

Co-investigators/Cooperators: Florida subtropical fruit processors; University of Florida. Potential: Florida Department of Plant Industry.

Work Plans for FY 92: Determine which cultivars are most resistant or are non-hosts using forced field, lab infestations of mango, orange, avocado and others. Evaluate natural infestation levels in packinghouse fruits.

II.A.2.c Armstrong, J.

CRIS 5320-43000-009-00D

SY: 1.5

Objective: Prove non-host status when possible for Hawaii-grown fruits (and U. S. mainland commodities when applicable) listed as fruit fly hosts, and develop quarantine parameters for fruits that are non-hosts during specific developmental stages.

Significance: Demonstration of non-host status precludes the need for quarantine treatment before export to the U. S. mainland or Japan, thereby opening new markets and expanding the Hawaii's agricultural and economic base. Non-host status precludes the need for treatment of U. S. mainland commodities in the event of fruit fly infestation. Successful non-host status designations for fruits exported to Japan and other foreign countries impacts balance of trade.

Justification: Regulatory - II.A, II.D, II.F.

Constraints: Acceptability of non-host status data and quarantine parameters by regulatory agencies and states/countries where non-host fruits will be marketed.

References:

- Armstrong, J. W., J. D. Vriesenga and C. Y. L. Lee. 1978. Resistance of pineapple varieties D-10 and D-20 to field populations of oriental fruit flies and melon flies. *J. Econ. Entomol.* 72(1):6-7.
- Armstrong, J. W., S. Tebbets and H. D. Nelson. 1981. Infestation studies of walnuts by Mediterranean fruit fly, Ceratitidis capitata. Walnut Research Reports (California industry reports). PP. 69.
- Armstrong, J. W., S. Tebbets and H. D. Nelson. 1981. Infestation studies of prunes by Mediterranean fruit fly, Ceratitidis capitata. Prune Research Reports (California industry reports). PP. 78.
- Armstrong, J. W., S. Tebbets and H. D. Nelson. 1981. Infestation studies of raisins by Mediterranean fruit fly, Ceratitidis capitata. Raisin Research Reports (California industry reports). PP. 115.
- Armstrong, J. W. and R. I. Vargas. 1982. Resistance of pineapple variety 59-656 to field populations of oriental fruit flies and melon flies. *J. Econ. Entomol.* 75(5):781-782.
- Armstrong, J. W. 1983. Infestation biology of three fruit fly species on Brazilian, William's and Valery cultivars of banana in Hawaii. *J. Econ. Entomol.* 76(3):539-543.
- Spitler, G. H., J. W. Armstrong and H. M. Couey. 1984. Mediterranean fruit fly host status of commercial lemon. *J. Econ. Entomol.* 77(6):1441-1444.
- Armstrong, J. W. 1991. "Sharwil" avocado: Quarantine security against fruit fly infestation in Hawaii. *J. Econ. Entomol.* 84(4). (In press).

Co-investigators/Cooperators: Hawaii tropical fruit industry and impacted growers.

Work Plans for FY 92: Initiate host status studies with "Bears" lime in the field and laboratory.

Also see Armstrong II.A.1.

B. Fly-Free Zones

Background - Fruit fly free commodities may be exported by eliminating tephritid populations from regions where they are endemic or have become established, or by reducing these populations to levels that the probability of infesting commercial hosts is negligible. Technology to reduce or eliminate populations such as sterile insect release, male annihilation, toxic baits and alternate host-plant destruction has been applied to establish fly-free zones against Anastrepha ludens in Texas and southern California, A. suspensa in citrus producing zones of Florida and for several Anastrepha species in Sonora and Baja California Norte in Mexico.

Needs - Methods to eliminate or drastically reduce populations of fruit flies are frequently too expensive or cause extensive environmental disruption or are otherwise unacceptable. Methods of maintaining these zones include a complex integrated system of detection, maintenance of quarantines and

integrated pest management. Better technology, attention to social issues and long term funding is necessary to establish or extend fly-free zones in Hawaii, Mexico and Florida.

1. Anastrepha

a. A. suspensa

b. A. ludens

2. Bactrocera (Dacus)

3. Rhagoletis

II.B.3 Yokoyama, V. Y.

CRIS 5302-43000-016-00D

SY: 0.2

Objective: Demonstrate that all stone fruits shipped to New Zealand from the San Joaquin Valley in California before July 1 of each year are free of walnut husk fly, Rhagoletis completa.

Significance: The New Zealand suspension of stone fruit shipments from California has resulted in the need for quarantine treatments to control the pest in exported fruit. Walnut husk fly has one generation per year and the adults emerge in midsummer. Emergence is predicted to occur after July 1 in the San Joaquin Valley. Establishment of a fly-free period prior to July 1 will eliminate the need for quarantine treatments of stone fruits shipped during this period. Implementation of a fly-free period will reduce postharvest handling costs and help preserve the export market for stone fruits in all Pacific Rim countries.

Justification: Industry - II.H.

Constraints: None.

References:

Yokoyama, V. Y., G. T. Miller and P. L. Hartsell. 1991. Pest-free period and methyl bromide fumigation for control of walnut husk fly (Diptera: Tephritidae) in stone fruits exported to New Zealand. J. Econ. Entomol. 84: (In press).

Co-investigators/Cooperators: California grower-shippers; California Cooperative Extension Farm Advisors; California County Agricultural Commissioners; California Department of Food and Agriculture; California Tree Fruit Agreement.

Work Plans for FY 92: Establish a walnut-husk fly-free period for the San Joaquin Valley in California.

C. Inspection/Interception Systems

Background - Prevention of introduction of fruit flies into non-infested but potentially suitable habitats is usually much more economical than elimination of populations after they have become established. Increased passenger traffic and commerce between fruit fly infested areas such as Hawaii and Latin America and potential habitats in the U.S. has increased the need for rapid, economical and effective means of detecting pests.

Needs - Means of detecting host material in baggage or mail such as chemical sensing equipment or use of dogs and other methods of reducing illegal imports are necessary. Research on ways to improve public cooperations in preventing exotic fruit fly introduction are also needed. Techniques such as genetic and immunological analysis are needed to determine the origin of exotic pests.

Also see Armstrong II.A.1.e.

1. Chemical Detectors

2. Physical Detectors

II.C.2 Calkins, C. O.

CRIS 6615-43000-006-00D

Also: I.E.1

SY: 0.2

Objective: Refinement and automation of the acoustical detector for monitoring of fruit or larval fruit fly infestations.

Significance: Large numbers of fruit (as much as 600/shipment) are sampled by cutting to ascertain the presence or absence of fruit fly larvae at points of embarkation. The acoustical device will detect extremely small larvae more efficiently than present methods. Also, the technique will not destroy the fruit in the process.

Justification: Industry - II.C.

Constraints: Access to embarkation ports where this sampling is being conducted. Development of robotics to

automate the sampling has not progressed because the area of use has not been established.

References:

Calkins, C. O. and J. C. Webb. 1988. Temporal and seasonal differences in movement of the Caribbean fruit fly larvae in grapefruit and the relationship to detection by acoustics. Fla. Entomol. 71:409-416.
Webb, J. C., D. C. Slaughter and C. A. Litzkow. 1988. Acoustical system to detect larvae in infested commodities. Fla. Entomol. 71:492-504.

Patent: Litzkow, C. A., J. C. Webb, S. Masuda. 1987. Acoustical detection of hidden insects.

Co-investigators/Cooperators: D. Shuman, ARS, Gainesville; R. Hickling and J. C. Webb, National Center for Physical Acoustics, Univ. of Mississippi; APHIS S&T International Services.

Work Plans for FY 92: To improve and test equipment in real world situations.

3. Social Research

4. ELISA Technique

II.C.4 Yokoyama, V. Y. CRIS 5302-43000-016-00D

SY: 0.1

Objective: Utilize a rapid immunoassay procedure that is commercially available to detect walnut husk fly, Rhagoletis completa, in stone fruits.

Significance: New Zealand suspended shipments of stone fruits from California in 1989 due to potential walnut husk fly infestations. A methyl bromide quarantine treatment and a pest-free shipping period are under development to control walnut husk fly in stone fruits shipped to New Zealand. Use of a rapid immunoassay procedure will help determine the presence of fruit fly infestations in exported fruit and reduce handling requirements during fruit inspection and certification.

Justification: Industry - II.C; Regulatory - II.C.

Constraints: None.

References:

Yokoyama, V. Y., G. T. Miller and P. L. Hartsell. 1991. Pest-free period and methyl bromide fumigation for control of walnut husk fly (Diptera: Tephritidae) in stone fruits exported to New Zealand. J. Econ. Entomol. 84: (In press).

Co-investigators/Cooperators: California Tree Fruit Agreement; California Department of Food and Agriculture; California grower-shippers.

Work Plans for FY 92: Evaluate the potential of an immunoassay procedure that is commercially available to detect walnut husk fly in stone fruits.

III. Control and Eradication

A. Chemical Pesticides (with and without attractants)

Background - The use of malathion in adult bait sprays in urban areas has been objected to by the general public. The objections may be more concerned with the aerial applications themselves, however. Diazinon has received some criticism and may be banned by EPA.

Needs - It may be possible to reduce the dose of malathion in the bait sprays substantially. Boric acid has been investigated as a replacement for malathion and shows some promise. It should be pursued if it appears feasible. A replacement for Diazinon soil drenches under infested trees is needed. Microbial toxicants or hormonal disruptors additions to a bait spray may replace malathion.

1. Soil Treatments

III.A.1 Purcell, M. F. CRIS 5320-43000-006-00D

Also: III.A.2.d

SY: 0.25 (Purcell), 0.05 (Jang)

Objective: Development of safer control alternatives (i.e. azadirachtin, cyromazine, entomophagous nematodes) for use in bait spray, ground treatment and other control applications for fruit flies.

Significance: Current chemicals used in control programs used by growers and in eradication programs have adverse effects on non-target species. Therefore, new chemicals should be sought which have minimal adverse effects on non-target species.

Justification: Regulatory - III.E.

Constraints: None.

References: None.

Co-investigators/Cooperators: R. Messing, Univ. of Hawaii, Kauai; J. Stark, Washington State University.
Potential: E. Jang, ARS, Hilo.

Work Plans for FY 92: Determine the toxicity of biological insecticides (e.g. neem, entomophagous

nematodes) against oriental, melon, Mediterranean fruit flies and fruit fly parasitoids.

Also see Vail III.A.2.d.

2. Bait Sprays

a. Microbials

III.A.2.a Mangan, R.

CRIS 6204-43000-003

Also: III.A.2.b

SY: 0.1

Objective: Develop more efficient adult suppression programs for fruit fly outbreaks such as new insecticides and new delivery techniques. Develop methods to use sterile flies as systems to deliver adult suppression agents to native populations.

Significance: Broad range sprays of nonspecific insecticide baits are unpopular with segments of the public and disrupt biocontrol systems. Sterile flies are known to select the same habitats as feral populations. Feeding on regurgitated fluids is an important aspect of inter-fly communication.

Justification: Industry - III.A, III.C, III.E, III.G, III.H; Regulatory - III.A, III.B, III.Y, III.HH, III.FFF.

Constraints: Public perception of what is dangerous and relatively risky. Lack of less toxic but equally effective insecticidal agents is a need. There is a need for disease or insecticidal agents which can be carried by released insects and delivered to target habitats before killing the released insect.

References: None.

Co-investigators/Cooperators: A. DeMilo, ARS, Beltsville; P. Vail, ARS, Fresno.

Work Plans for FY 92: Determine, for caged populations of A. ludens, the degree of transfer of regurgitates from a group of released, dye-fed flies to a target population. Make preliminary screening of possible agents and methods of feeding.

III.A.2.a Jang, E.

CRIS 5320-22000-005-00D

Also: III.A.2.d; IV.B.1; IV.B.3; IV.B.5

SY: 0.1

Objective: Development of alternative toxicants for use in control of fruit flies.

Significance: Public concern over the use of insecticides such as malathion for wide spread aerial spraying and in bait sprays used in eradication programs require a search for a suitable replacement. Novel toxicants such as microbials which may be species specific are needed.

Justification: Regulatory - I.F, III.A, III.B, III.C, III.E, III.II.

Constraints: Identification of novel control compounds such as microbiologicals will require an intensive effort in basic physiology to uncover fruit fly vulnerabilities.

References: None.

Co-investigators/Cooperators: P. Vail, ARS, Fresno.
Potential: S. Krasnoff, ARS, Ithaca.

Work Plans for FY 92: Isolate potential vectors from fruit flies in cooperation with co-investigators and cooperators. Initiate lab bioassays.

III.A.2.a Krasnoff, S. B. CRIS 1907-22000-006-00D

SY: 1.0

Objective: To discover alternative toxicants for adult fruit fly control by screening secondary metabolites of entomopathogenic fungi.

Significance: Eradication campaigns spearheaded by aerial spraying of malathion have resulted in a public outcry against widespread use of traditional pesticides. The search for novel natural products with insecticidal activity, besides offering the hope of finding new tools for resistance management, has the added payoff of addressing public concerns about the environmental impact and human toxicity of synthetic insecticides. Insect pathogens have become the focus of attempts to discover new, highly selective insecticidal agents. Entomopathogenic fungi, important players in this arena, produce a variety of novel compounds with insecticidal activity. The ARS collection of entomopathogenic fungi (ARSEF), the largest of its type in the world, represents a vast untapped resource of potentially useful natural products. Systematic screening of this collection,

ongoing in our laboratory, has already yielded several extracts with promising activity and should lead to the isolation and identification of novel compounds with high target-specificity against medfly and other tephritid fruit fly pests.

Justification: Regulatory - III.A.

Constraints: This research is funded by a two-year temporary transfer of funds from CRIS Nos. 5320-24000-004-00D, 5320-43000-007-00D, 6631-43000-004-00D and 6631-43000-005-00D. These funds will terminate September 30, 1991.

References:

- Krasnoff, S. B. and S. Gupta. 1991. Identification and directed biosynthesis of efraeptins in the fungus Tolypocladium geodes (Deuteromycotina: Hyphomycetes). J. Chem. Ecol. 17:1953-1962.
- Krasnoff, S. B., S. Gupta, R. J. St. Leger, J. A. A. Renwick and D. W. Roberts. 1991. Insecticidal and antifungal properties of the efraeptins: Toxins of the fungus Tolypocladium niveum. J. Invert. Pathol. 58:180-188.
- Gupta, S., S. B. Krasnoff, N. L. Underwood, J. A. A. Renwick and D. W. Roberts. 1991. Isolation of beauvericin as an insect toxin from Fusarium semitectum and Fusarium moniliforme var. subglutinans. Mycopathologia, in press.
- Gupta, S., Krasnoff, S. B., Roberts, D. W., Renwick, J. A. A., Brinen, L. and Clardy, J. 1991. The structures of the efraeptins-Potent inhibitors of mitochondrial ATPase from the fungus Tolypocladium niveum. J. Am. Chem. Soc. 113:707-709.

Co-investigators/Cooperators: R. T. Cunningham, ARS, Honolulu. Potential: E. Jang, ARS, Hilo; J. Sharp, ARS, Miami.

Work Plans for FY 92: We will produce and screen extracts of up to four new fungal isolates per week. Dose/response bioassays will be conducted on active crude extracts to determine optimal dose for pursuing isolation of active compounds. Preliminary chromatographic purification (silica gel flash chromatography, HPLC) of at least one active isolate and testing of active fractions will be conducted.

III.A.2.a Vail, P. V. CRIS 5302-43000-018

Also: III.D.1.c

SY: 0.1

Objective: Few viruses have been isolated from economically important fruit fly species. We propose to isolate, characterize, determine infectivity and potential of viruses infectious to fruit flies as microbial control agents. These organisms could be used

for direct control or in inoculative type releases to reduce the reproductive potential of fruit flies. Existing and newly developed bait/attractants would provide the vehicle for dissemination.

Significance: Despite the insecticidal activity of presently used baits to medfly adults, the main objective of bait sprays is and should be the reduction of the reproductive potential of medfly (fruit fly) populations. Although presently a result of acute toxicity, sublethal or chronic effects could have the same results (i.e., reduced reproductive potential). The availability of baits/attractants could provide an excellent delivery system for microbial control agents. Of the few viruses thus far isolated from fruit flies, many host effects have been noted: infection of both larval and adult stages; reduction in egg laying; infection of numerous major tissues; and large quantities of virus in feces (may provide for horizontal and vertical transmission and production in *in-vitro* systems). The extended preoviposition period for medfly and other tropical fruit flies should provide enough time for the viruses to have an effect. These viruses, along with a concentrated effort to identify other viruses infectious to fruit flies could provide a biorational alternative that could be used for immediate population reduction and/or to lower fruit fly populations over extended periods once introduced into the populations.

Justification: Industry - III.A, III.E; Regulatory - III.A, III.B.

Constraints: Plans have been made to conduct this research at a low level over the next several years in cooperation with the USDA-ARS, Hilo, Hawaii laboratory and a microbiologist at U. C. Davis, California. Other constraints include the need for safety testing of candidate viruses.

References:

- Bashiruddin, J. B., J. L. Martin and C. Reinganum. 1988. Queensland fruit fly virus, a probable member of the Picornaviridae. Arch. Virol. 100:1-74.
- Manousis, T., M. K. Arnold and N. F. Moore. 1988. Electron microscopical examination of tissues and organs of Bactrocera oleae infected with cricket paralysis virus. J. Invertebr. Pathol. 51:119-125.
- Moawed, S. M. and N. F. Shehat. 1987. Effects of NPV of the Egyptian cotton leafworm Spodoptera littoralis (Boisd) on the Mediterranean fruit fly Ceratitidis capitata (Wied.). Insec. Sci. Appl. Oxford 8(3):365-368.
- Moussa, A. Y. 1978. A new cytoplasmic inclusion virus from Diptera in the Queensland fruitfly, Bactrocera tryoni (Frogg) (Diptera: Tephritidae). J. Invertebr. Pathol. 32:77-78.

Plus, N., J. C. Veyrunes and R. Cudlorao. 1984. Endogenous viruses of Ceratitidis capitata Wied. "JRC Ispra" strain, and of C. capitata permanent cell lines. Ann. Virol. (Institut Pasteur) 132E:91-100.

Co-investigators/Cooperators: E. B. Jang, ARS, Hilo; J. Manning, Dept. of Microbiology, Univ. of California, Davis; APHIS S&T, Hyattsville. Potential: R. Mangan, ARS, Weslaco.

Work Plans for FY 92: 1) Obtain known viral pathogens of fruit flies; 2) Conduct initial screening of pathogens in vivo and in vitro at ARS Hawaii and Fresno.

Also see Lindegren III.D.1.d.

b. Hormonal

III.A.2.b Moreno, D.

CRIS 6204-43000-003-00D

SY: 0.2

Objective: Find a more environmentally acceptable bait than the malathion bait to control fruit flies.

Significance: Historically the malathion bait has been used successfully to control fruit flies around the world. However, adverse effects of its application have been well documented. Aside from social effects, the malathion bait kills natural enemies of other insect pests. The absence of biological control causes pest populations to increase exponentially without natural regulation. This in turn tends to cause producers to spray more insecticides to reduce the increasing populations. The finding of a more specific compound(s) or pathogens that would sterilize, disorient, or kill the Mexican fruit fly would be highly beneficial environmentally.

Justification: Industry - III.A, III.C; Regulatory - III.A.

Constraints: The testing for novel compounds with fruit flies is laborious and time consuming. Basically, we only have the adult stage to work with because all of the other target stages are inside plant tissues and are inaccessible for experimentation or control. Careful testing is necessary to define the specific effect for each novel compound if nothing is known. The long life of the adult insect precludes that only short-period testing be conducted. If a sterilant is sought, then we must make sure that the effect is for life rather than for only a couple of weeks. If a successful compound is

found in the laboratory, then it must also be tested in field cages (Mexico) and if successful here, it must be tested with feral insects in a natural setting.

References: None.

Co-investigators/Cooperators: R. L. Mangan, ARS, Weslaco, Texas; A. B. DeMilo, ARS, Beltsville, Maryland.
Potential: APHIS S&T, Mission, Texas.

Work Plans for FY 92: Obtain candidate insect growth regulators. Determine their effect on adult insects. Develop a narrow testing range. Evaluate specific effects related to mortality, morphogenic changes, behavior, fecundity and residual effects. This will take considerable time due to the long life cycle of the Mexican fruit fly (38 days from egg to egg at 26.7°C and a half life of 60 days for adults at the same temperature). Field cage testing may not be feasible in one year.

Also see Mangan III.A.2.a.

c. Malathion

(Investigated by APHIS)

0.1 Dose

0.2 Replacements/Inorganics-Boron/Pyrethroids

d. Other

III.A.2.d Vail, P. V.

CRIS 5302-43000-018

Also: III.A.1

SY: 0.1

Objective: To determine the biological activity of non-toxic high molecular weight proteins on the longevity and reproductive potential of medfly and other fruit fly species.

Significance: This class of compounds has not been investigated for biological activity against insects. An annihilation method not dependent upon the use of chemical insecticides is needed. The compounds we plan to test are readily available off the shelf or as mixtures of several proteins and are not known to have undesirable, safety or environmental effects. Together

with baits or attractants available now or in the future, it is possible that a high degree of insecticidal activity can be obtained without the undesirable characteristics now associated with bait sprays.

Justification: Industry - III.A, III.E; Regulatory - III.A, III.B.

Constraints: Small scale testing is planned in cooperation with ARS, Hawaii. Laboratory testing will be limited by other program requirements, the availability of funds, personnel and travel needs. Field cage or field testing of candidate materials will not be conducted unless travel and personnel funding are made available.

References: None.

Co-investigators/Cooperators: E. Jang, ARS, Hilo; L. Aung, ARS, Fresno; APHIS S&T, Hyattsville. Potential: R. Mangan, ARS, Weslaco.

Work Plans for FY 92: Selected high molecular weight proteins will be screened for activity against adult medflies.

Also see Purcell III.A.1, Jang III.A.2.a.

B. Male/Female Annihilation

Background - Male annihilation has been used as a standard control measure for melon fly and oriental fruit fly for several years. It has not been tried with other species.

Needs - Female annihilation seems feasible with the papaya fruit fly. Male annihilation of medfly may be feasible with long-lasting formulations of ceralure or other isomers of trimedlure. The discovery and use of pheromones and appropriate traps may make it feasible as a control measure for some of the Anastrepha species. A replacement for methyleugenol/naled may be required in the future.

1. Male Annihilation

a. Oriental Fruit Fly

III.B.1.a Purcell, M. F. CRIS 5320-43000-006-00D

SY: 0.25 (Purcell), 0.05 (Cunningham)

Objective: Determine the efficiency of methyleugenol traps in eradication programs for the oriental fruit fly.

Significance: Currently it is not known the range in which methyleugenol attracts oriental fruit flies and what percentage of the populations is attracted to these traps. This information would help provide useful guidelines for efficient placement of traps, and would help in making decisions for balancing the costs of traps with the efficiency of these traps.

Justification: Regulatory - I.M.

Constraints: None.

References: None.

Co-investigators/Cooperators: R. Cunningham, ARS, Hilo.

Work Plans for FY 92: Determine the efficiency of methyl eugenol traps in eradication programs for the oriental fruit fly (team effort with Nic Liquido (USDA, Hilo), Russell Messing (Univ. Hawaii), Tim Wong (USDA, Honolulu) and R. Plant (UC, Davis).

III.B.1.a Vargas, R.

CRIS 5320-22000-014-00D

SY: 0.25 (Vargas), 0.25 (Spencer)

Objective: Expand present male annihilation suppression/eradication program to encompass all major habitats (wild host, endemic, forest agricultural, and urban areas) on Kauai.

Significance: During the past year we have been involved in large-scale pilot tests to suppress populations of the oriental fruit fly and melon fly on the island of Kauai. Although the use of bucket traps has effectively reduced populations, these traps have been costly and laborious. We would like to shift to applications of minugel and dibrom. Immediate field testing of convenient environmentally-sound male annihilation procedures in the field is needed for eradication of fruit flies in Hawaii.

Justification: Regulatory - II.B, III.H, III.FF.

Constraints: Experimental Use Permits (EUP's) will have to be modified.

References:

- Vargas, R. I., J. D. Stark and T. Nishida. 1989. Abundance, distribution and dispersion indices of the oriental fruit fly and melon fly (Diptera: Tephritidae) on Kauai, Hawaiian Islands. J. Econ. Entomol. 82:1609-1615.
- Vargas, R. I., J. D. Stark and T. Nishida. 1990. Population dynamics, habitat preference and seasonal distribution patterns of oriental fruit fly and melon fly (Diptera: Tephritidae) in a agricultural area. Environ. Entomol. 19:1820-1828.

Co-investigators/Cooperators: J. Spencer and M. Purcell, ARS, Kapaa; R. Messing, M. Kido, C. Myles and J. Harrison, Univ. of Hawaii.

Work Plans for FY 92: Expand methyl eugenol and cuelure mass trapping densities within Molokai experimental area. Begin applications to use minugel in Hawaii fruit fly eradication programs.

0.1 Alternatives for Methyleugenol-Naled

Also see DeMilo I.B.2.b.

b. Melon Fly

c. Malaysian Fruit Fly

III.B.1.c *Liquido, N. J.* CRIS 5320-22000-005-00D
5320-22000-012-00D

SY: 0.2

Objective: Development of an effective male annihilation system for Malaysian fruit fly based on the newly discovered attractant, latilure (alpha ionol).

Significance: A male annihilation system would provide a convenient and economical eradication technology in the event of accidental introduction of Malaysian fruit fly in the mainland U. S. Furthermore, male annihilation would provide an additional technology to eradicate established population of Malaysian fruit fly in Hawaii.

Justification: Regulatory - I.A, III.F.

Constraints: An EUP is needed to carry out a pilot test, which probably will require a Tier I toxicological data. The cost of Tier I testing would be between \$20,000 - \$50,000.

References: None.

Co-investigators/Cooperators: R. Cunningham, ARS, Hilo; B. Leonhardt, ARS, Beltsville. Potential: Hawaii Dept. of Agriculture; University of Hawaii; APHIS S&T.

Work Plans for FY 92: Develop delivery system for latilure; determine behavioral responses of B. latifrons to latilure as affected by age, mating status and nutritional condition.

d. Mediterranean Fruit Fly

0.1 Sticky boards

III.B.1.d.0.1 *Liquido, N. J. CRIS* 5320-22000-012-00D
5320-22000-005-00D
5320-22000-013-00D

Also: III.B.1.d.0.2-0.4

SY: 0.1

Objective: Development of an effective male annihilation system for Mediterranean fruit fly based on the newly discovered attractant, ceralure.

Significance: An effective and economical male annihilation system for Mediterranean fruit fly may replace the sterile insect release technology as the system of choice for eradicating periodic invasions of Mediterranean fruit fly in the mainland U. S. Furthermore, ceralure-based male annihilation system might be used to eradicate established populations of Mediterranean fruit fly in many parts of the world, including Hawaii.

Justification: Industry - III.B, III.G; Regulatory - I.A, I.B, I.D, I.I, III.F.

Constraints: A convincing pilot test on efficacy must be carried out in at least 1 km² experimental area. This requires a great deal of manpower and material such as ceralure which costs \$1,000 per kg. There is also difficulty in obtaining sufficient numbers of usable large plots for replicated experiments. A single pilot test with four replicates will cost \$32,000 for the lure alone.

References: None.

Co-investigators/Cooperators: R. Cunningham, ARS, Hilo; APHIS S&T, Guatemala; B. Leonhardt, ARS, Beltsville; APHIS S&T, Honolulu; Univ. of Hawaii; Hawaii Dept. of Agriculture. Potential: CDFA.

Work Plans for FY 92: Initiate preliminary work on efficient delivery system (type of trap; lure-toxicant dosage formulation; lure dispenser, etc.) for ceralure.

0.2 Plastics

Also see Liquido III.B.1.d.0.1.

0.3 Min-U-gel

Also see Liquido III.B.1.d.0.1.

0.4 Formulations of attractants

III.B.1.d.0.4 Leonhardt, B. CRIS 1275-22000-089-00D

SY: 0.3

Objective: Develop a controlled-release delivery system for the use of synthetic attractants (ceralure, trimedlure, or isomer C of TML) in a male annihilation program for control of the Mediterranean fruit fly.

Significance: Such a system, if proved effective and economical, could be used in the eradication of periodic infestations of the Mediterranean fruit fly. It might also be used as a tool in medfly barrier zone programs.

Justification: Industry - III.A; Regulatory - I.F.

Constraints: Biological efficacy must be demonstrated in a relatively large field plot. Chemical integrity and effective release rate of the lure must be preserved over a sufficient duration to make the system economical.

References: None.

Co-investigators/Cooperators: R. Cunningham and N. Liquido, ARS, Hilo; APHIS S&T, Guatemala; Commission Moscamed, Guatemala; Hawaii Dept. of Agriculture.

Potential: APHIS S&T, Honolulu, R. Messing, University of Hawaii.

Work Plans for FY 92: Measure release rates from ceralure/stickum mixtures; compare ceralure vs. TML-C.

III.B.1.d.0.4 Leonhardt, B. CRIS 1275-22000-089-00D

SY: 0.1

Objective: Develop alternate formulations of trimedlure (TML) for use in detection or control programs.

Significance: The duration of high attractivity of the polymeric plug dispenser could be improved by increasing the amount of TML per dispenser or by using just TML-C isomer. Also, containing TML (or just TML-C isomer) polymeric beads could be mixed with adhesive or suspended in a gel for use in male annihilation of medflies; such formulations might be less expensive and as effective as formulated ceralure in male annihilation programs.

Justification: Regulatory - I.I.

Constraints: An increase in TML content per trap for medfly detection would increase the initial cost but would prolong trap effectiveness. The use of TML-C would require separation of this component (ca.40% abundant) from bulk TML.

References: None.

Co-investigators/Cooperators: R. Cunningham and N. Liquido, ARS, Hilo; R. Rice, Univ. of California, Davis; AgriSense, Fresno. **Potential:** California Department of Food and Agriculture; APHIS S&T.

Work Plans for FY 92: Obtain bead formulations of TML and/or TML-C; test effect of Naled insecticide with TML in plugs.

Also see Liquido III.B.1.d.0.1.

0.5 Attractants

III.B.1.d.0.5 DeMilo, A. B. CRIS 1275-22000-089

SY: 0.1

Objective: Provide pure isomers or blends of isomers of trimedlure (TML) and ceralure for evaluation in the male annihilation technique.

Significance: TML-C is the most active and the least volatile isomer in trimedlure, and this isomer crystallizes from TML blends with a high C content. These reasons make TML-C a prime candidate for application in the male annihilation method to control the medfly. Like TML-C, ceralure and probably its active B₁ isomer are less volatile than TML. Therefore, the B₁ isomer along with ceralure blends containing a high B₁ content should also be evaluated for their use in the male annihilation program (some of this work is in progress).

Justification: Industry - I.B, III.A, III.B, III.C;
Regulatory - I.D.

Constraints: The ability to use any of these attractants in the male annihilation program must be demonstrated on a large scale. Factors such as efficacy, volatility, stability and chemical compatibility in formulated products may deny their successful use in the male annihilation method.

References: None.

Co-investigators/Cooperators: R. Cunningham, ARS, Hilo;
B. Leonhardt and J. Warthen, ARS, Beltsville.

Work Plans for FY 92: Methods to rapidly, economically and efficiently isolate TML-C from commercial mixtures will be explored. Methods to increase B₁ content in ceralure will be explored. Blends high in TML-C and/or Ceralure B₁ content will be supplied for bioassay and formulation studies.

e. Anastrepha

Also see DeMilo I.B.5.a.

2. Female Annihilation

Also see Jang 1.C.2.c.

a. Oriental Fruit Fly

b. Papaya Fruit Fly

III.B.2.b Landolt, P. J. CRIS 6615-22000-009-00D

SY: 0.05

Objective: Development of control technology for the papaya fruit fly based on female annihilation using the male pheromone and trap. Optimization of efficacy of combined chemical attractant and annihilation technology with cost reduction measures.

Significance: Control methods are not currently available for this species in commercial papaya groves in Florida or in the Caribbean Basin. Success with this fruit fly should help with efforts to determine similar technology later for other species of Tephritidae.

Justification: Regulatory - I.D, III.A. This species has been a useful model for developing research methods

and approaches to use with the medfly pheromone and will continue as such.

Constraints: The papaya fruit fly population in Florida fluctuates wildly and is susceptible to severe reductions from freezing temperatures. Tests have been held up until the population recovers from the December 1989 freeze.

References: None.

Co-investigators/Cooperators: R. Heath, ARS, Gainesville.

Work Plans for FY 92: None. No flies are available.

c. Mediterranean Fruit Fly

III.B.2.c Heath, R. R. CRIS 6615-22000-008-07R

Also: I.D.2

SY: 0.1

Objective: Continue the development of an attracticide system for fruit flies using an applicatory combination of visual cues, attractants and toxicants.

Significance: This research will provide an alternative to aerial bait sprays currently being used. Such systems will be tested as possible annihilation systems for the medfly, Caribbean and Mexican fruit fly. The attracticide system will also provide enhanced monitoring with increased trapping efficacy with currently used parapheromones such as trimedlure.

Justification: Industry - I.A, I.B, III.A, III.B, III.C, III.G; Regulatory - I.A, I.B, I.D, I.H, I.M, III.A, III.B, III.D, III.F, III.I, III.QQ.

Constraints: None.

References:

Heath, R. R., D. L. Chambers, J. H. Tumlinson and P. J. Landolt. 1990. A controlled release formulation of trimedlure isomer C and its attractiveness to the Mediterranean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 83(3):819-822.

Co-investigators/Cooperators: P. Landolt, N. D. Epsky and J. Sivinski, ARS, Gainesville; APHIS S&T, Guatemala.

Work Plans for FY 92: Determine which combinations of visual cues, attractants and toxicants provide the greatest potential for further development of an attracticide system. Use of TML-C crystals as an attractant in the attracticide system will be evaluated.

d. Anastrepha

e. Spacing and Dosage Information

C. Autocidal Control (SIT)

1. Rearing

Background - Mass rearing methods have been developed for the medfly, melon fly, oriental fruit fly, Caribbean fruit fly and the Mexican fruit fly.

Needs - More efficient rearing procedures are needed for these species especially in the areas of nutrition and automation. Mass rearing techniques have yet to be devised for the Malaysian and the West Indian fruit flies. There has been no success in mass rearing any of the non-resident Anastrepha species. If any of these invade the U.S., the only weapon that could be used for eradication would be bait sprays. Use of a method of genetic sexing to produce only male flies would reduce the cost of rearing significantly and may increase the efficiency of the sterile release method because only males would be released. Process quality control methods need to be improved.

III.C.1 Hennessey, M. K. CRIS 6631-43000-007-00D

SY: 1.5

Objective: Maintenance, improvement, characterization of Caribbean fruit fly colony.

Significance: Many experiments depend on large quantities of flies demonstrating consistent qualities of fecundity and vitality. Improvement will include cutting rearing costs. Characterization will include genetic identification and determining host acceptability of various strains used in non-host and ecological experiments.

Justification: Industry - III.D, III.G; Regulatory - III.L, III.M, III.Q, III.R.

Constraints: Depends in part on cooperation of molecular geneticist on station.

References: None.

Co-investigators/Cooperators: Potential: Other Caribbean fruit fly rearing facilities at the University of Florida, Florida Department of Plant Industry, and ARS, Orlando.

Work Plans for FY 92: Determine isozyme differences or DNA strand differences for various strains and correlate with behavior, host-acceptance. Develop less expensive mass rearing diet with higher efficiency.

a. Diet

III.C.1.a Jang, E.

CRIS 5320-22000-011-00D

Also: IV.B.1; IV.B.3; IV.B.5

SY: 0.1

Objective: Improved diets for mass-rearing of fruit flies.

Significance: Variation in the quality of raw materials used in mass-rearing of fruit flies makes it difficult to insure consistent product yield and fly quality. Large scale eradication programs such as SIT are therefore vulnerable to rearing "crashes" which can paralyze operations.

Justification: Industry - III.D; Regulatory - III.L, III.Q.

Constraints: None.

References:

- Jang, E. B. 1986. Effects of Niacin Deficiency on Growth and Development of the Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann). J. Econ. Entomol. 79:558-561.
- Jang, E. B. 1990. Physiological and biochemical approaches in the development of technology for eradication of fruit fly pests. In Proc. Symp. on Eradicating Alien Fruit Flies from the Unique Hawaiian Environment. Poipu, Kauai.

Co-investigators/Cooperators: H. Chan, ARS, Hilo; D. McInnis and H. Chang, ARS, Honolulu; APHIS S&T, Waimanalo. Potential: N. Tanaka, CDFA.

Work Plans for FY 92: I.D. contaminants in wheat mill responsible for "crashes." Improve corn cob based diet for medfly.

III.C.1.a Chan, H.

CRIS 5320-22000-011-00D

SY: 0.5

Objective: Development of methods to control the acetic acid production in wheat-based fruit fly diets.

Significance: The production of acetic acid caused by the ubiquitous fermenting organisms in the wheat mill poses an environmental workplace hazard and causes extensive corrosion of equipment. In addition the high levels of acetic acid may be detrimental to larval development. Methods for controlling the acid production would ameliorate the work hazard reducing worker discomfort and possibly improve fly production.

Justification: Industry - III.D; Regulatory - III.L, III.Q.

Constraints: A convincing demonstration of the effectiveness of the processed/reformulated diet must be carried out at the full production level. This will require cooperation and coordination with ARS and APHIS rearing groups and allocation of resources.

References:

Chan, H. T., Jr., J. D. Hansen and S. Y. T. Tam. 1990. Larval diets from different protein sources for Mediterranean fruit flies. J. Econ. Entomol. 83:1954-1958.

Co-investigators/Cooperators: E. Jang, ARS, Hilo; APHIS S&T, Waimanalo; H. Chang, ARS, Honolulu.

Work Plans for FY 92: Continue research on controlling acetic acid production at the production level and relate effects of Ph control on insect production and insect quality.

*III.C.1.a Chan, H. T.
Jang, E. B.*

CRIS 5320-22000-011-00D

SY: 0.5 (Seo), 0.5 (Chan), 0.1 (Jang)

Objective: Develop mass rearing procedures for Malaysian fruit fly.

Significance: Presently with the exception of insecticides, no area-wide eradication technique exists for eradication of Malaysian fruit fly. Development of mass rearing techniques are essential for development of the SIT approach that would offer an environmentally sound method for area-wide eradication important to both Hawaii and U. S. mainland areas susceptible to fruit fly introductions.

Justification: Industry - III.H; Regulatory - III.D, III.M, III.O.

Constraints: First, mass rearing methods need to be researched and developed; second, consistent large scale production of high quality insects needs to be demonstrated at levels of 25 million per week in a pilot test.

References:

- Vargas, R. I. and T. Nishida. 1985. Survey for Dacus latifrons (Diptera: Tephritidae). J. Econ. Entomol. 78:131-134.
- Vargas, R. I. and T. Nishida. 1985. Life history and demographic parameters of Dacus latifrons (Diptera: Tephritidae). J. Econ. Entomol. 78:1242-1244.
- Vargas, R. I. and S. Mitchell. 1987. Two artificial larval diets for rearing Dacus latifrons (Diptera: Tephritidae). J. Econ. Entomol. 80(6): 1337-1339.
- Vargas, R. I., S. Mitchell, B. Fujita and C. Albrecht. 1990. Rearing techniques for Dacus latifrons (Diptera: Tephritidae). Proc. Hawaii Entomol. Soc. 30:61-66.

Co-investigators/Cooperators: S. Seo and H. Chang, ARS, Honolulu; APHIS S&T, Hawaii Sterile Fruit Fly Facility.

Work Plans for FY 92: Complete research and development on Malaysian fruit fly mass rearing techniques.

0.1 Nutritional Needs

III.C.1.a.0.1 Jang, E. CRIS 5320-22000-011-00D

Also: IV.B.1; IV.B.3; IV.B.5.a

SY: 0.1

Objective: Identify nutritional requirements of fruit flies and their parasites.

Significance: The precise nutritional requirements of fruit flies and their parasites are largely unknown. Identification of nutritional requirements would facilitate development of improved diets for mass rearing of fruit flies and "in vitro" methods for mass rearing of fruit fly parasites.

Justification: Industry - III.D, III.F; Regulatory - III.L, III.Q.

Constraints: None.

References:

Jang, E. B. 1986. Effects of Niacin Deficiency on Growth and Development of the Mediterranean fruit fly, Ceratitidis capitata (Wiedemann). J. Econ. Entomol. 79:558-561.

Co-investigators/Cooperators: H. Chan, ARS, Hilo.

Potential: P. Lawrence, Univ. of Florida, Gainesville.

Work Plans for FY 92: Analysis of current diets for nutritional value. Investigate fruit fly nutritional bioassays.

0.2 Recycling

III.C.1.a.0.2 Chan, H. CRIS 5320-22000-011-00D

SY: 0.5

Objective: Develop technology for recycling spent fruit fly diets.

Significance: Spent fruit fly media poses a waste disposal/environmental problem. Recycling diet material would reduce costs of waste disposal and also recover diet material.

Justification: Industry - III.D; Regulatory - III.L, III.Q.

Constraints: Basic knowledge on rates of nutrient uptake and utilization needs to be established in order to fix the replenishment of nutrients. Knowledge of the existence of toxic metabolic wastes and byproducts are also lacking.

References:

Chan, H. T., Jr., J. D. Hansen and S. Y. T. Tam. 1990. Larval diets from different protein sources for Mediterranean fruit flies. J. Econ. Entomol. 83:1954-1958.

Co-investigators/Cooperators: E. Jang, ARS, Hilo; APHIS S&T, Waimanalo; H. Chang, ARS, Honolulu.

Work Plans for FY 92: Initiate research on the feasibility of recycling spent fruit fly diets.

0.3 Automation

0.4 Quality Control

III.C.1.a.0.4 Calkins, C. O. CRIS 6615-22000-009-00D

Also: III.C.2.a; III.C.2.b

SY: 0.4

Objective: Develop quality control technology for medflies and Caribbean fruit flies in the laboratory and the field. Advise on quality control programs for other species.

Significance: In sterile release programs, it is essential that the sterile released flies are not only competitive but are reproductively compatible with the target population. Methods to determine easily this compatibility are necessary to assure that the released flies will be effective. Technology to determine the effectiveness of the flies after their release such as sperm markers, demographic profiles, etc. need to be developed to serve as monitors during the eradication program.

Justification: Regulatory - III.L, III.O, III.S, III.U, III.V, III.WW.

Constraints: Access to wild and reared populations of medflies from several geographic areas are needed for mating studies.

References:

- Calkins, C. O. and T. R. Ashley. 1989. The impact of poor quality of mass-reared Mediterranean fruit flies on the sterile insect technique used for eradication. *J. Appl. Entomol.* 108:401-408.
- Calkins, C. O. 1989. Lekking behavior in fruit flies and its implications for quality assessments. PP 135-139 *In*: R. Cavalloro (Ed.) *Fruit flies of economic importance 87*. Proc. CEC/IOBC Intl. Sym., Rome, April 7-10, 1987. A. A. Balkoma, Rotterdam.
- Calkins, C. O. 1989. Quality control. PP 153-165 *In*: A. S. Robinson and G.H.S. Hooper (Eds.) *World Pest Crops: Fruit Flies, their biology, natural enemies and control*. Vol. 3B. Elsevier Publ., Amsterdam.
- Draz, K. A. A. and Calkins. 1989. Measuring and monitoring flight ability of *Anastrepha suspensa* (Loew) under laboratory conditions. 3rd Nat. Conf. of Pests and Dis. of Veg. & Fruits in Egypt and Arab Countries. Ismalia, Egypt.

Co-investigators/Cooperators: APHIS S&T, Guatemala; R. Cunningham, ARS, Hilo; Miller, Michigan State Univ.; J. Sivinski, ARS, Gainesville; R. Baranowski, Univ. of Florida, Gainesville.

Work Plans for FY 92: a) Test mass-reared populations with wild populations in Guatemala. Arrange for shipments of sterile flies from Hawaii to Guatemala to be tested for compatibility with mass-reared flies and wild flies. b) Monitor quality of mass-reared Caribbean fruit flies in the laboratory and field.

b. Establish Rearing Techniques for Other Species

III.C.1.b Hansen, J.D. CRIS 6631-43000-005-00D

SY: 0.2

Objective: Development of effective rearing methods for the papaya fruit fly.

Significance: A laboratory colony would be a reliable source of test insects year round. Hence, research could be conducted involving quarantine treatments, behavior and development of appropriate fly populations.

Justification: Industry - II.E, II.G; Regulatory - II.A.

Constraints: The papaya fruit fly is very host specific, feeding only on the endosperm, the testa, and the sarcotesta within the seed cavity of immature papayas. Dietary components that adequately substitute the nutritional quality have not been identified. Compounds that naturally occur in very small quantities may be important in promoting certain behaviors.

References: None.

Co-investigators/Cooperators: J. E. Peña, University of Florida, Homestead.

Work Plans for FY 92: Examine a variety of different diets for growth and development. Criteria that will be measured are: 1) pupal weight, 2) percent pupation, 3) emergence and 4) percent cohort survival.

0.1 A. obliqua

III.C.1.b.0.1 Moreno, D. S. CRIS 6204-43000-003-00D

SY: 0.2

Objective: Develop an optimum artificial diet for the West Indian fruit fly and determine its biology.

Significance: The West Indian fruit fly is surfacing as perhaps the most important fruit fly in mangos in Mexico and other mango exporting countries such as Haiti, Honduras, Costa Rica, Jamaica and others. The United States is currently importing great quantities of mangos from these countries. Thus, the fly represents a serious potential threat to U.S. Agriculture. Yet, not much is known about this fruit fly. So far, little or no success has been obtained in trying to rear this fruit fly in the laboratory. If we are to mass-rear this fruit fly and develop a SIT program for it, we must focus on its basic biology and rearing procedures.

Justification: Industry - III.D; Regulatory - III.M.

Constraints: The most difficult part of establishing the West Indian fruit fly on a meridic diet in the laboratory has been achieved. Our present colonies have been on a meridic diet for more than 10 generations and appear to be in good condition. However, we have not developed the methodology that would allow us to mass rear the fly. There are a number of basic biological studies that have to be performed in order to manipulate the fly colony towards mass production. The life cycle of the West Indian fruit fly is about the same as that for the Mexican fruit fly and consequently it will take time to achieve the desired goals.

References: None.

Co-investigators/Cooperators: APHIS S&T, Mexican Fruit Fly Rearing Facility, Mission, Texas. **Potential:** J. L. Leyva, Chapingo Colgio de Postgraduados, Texcoco, Mexico.

Work Plans for FY 92: Our current artificial diet still requires the incorporation of fresh fruit in order to rear the larvae. Our mature third instar pupal rate of return is about 12% based on seeding of first instar larvae. We will be testing a number of ingredients that we are currently acquiring to improve upon our present diet. Our goal is to develop a suitable diet without fresh fruit for A. obliqua the first year. Part of the evaluation will be to determine if the colony will perpetuate itself at the same density or increases over a period of six generations (about seven months).

0.2 A. fraterculus

c. Strain Selection

0.1 Mutation selection

III.C.1.c.0.1 McInnis, D. CRIS 5320-22000-011-00D

Also: III.C.1.c.0.2

SY: 0.2

Objective: Development and mass-production of genetically improved strains of tephritid fruit flies.

Significance: Genetic sexing strains promise to improve the efficiency of sterile insect programs through males-only releases or some other form of altered sex-ratio fly release. Preliminary laboratory and field studies of other medfly strains based upon quantitative selection of desired characteristics have yielded encouraging results (e.g. genetically large flies showing superior fitness traits). Genetic engineering using molecular biological techniques also promises to deliver new genetically altered strains for evaluation.

Justification: Regulatory - III.O, III.S, III.V, III.YY, III.ZZ, III.BBB.

Constraints: Maintenance of genetic purity of new strains, especially when mass-produced. Also, for any genetically engineered strain using exogenous DNA, the real chance of failure to achieve transformation must be considered.

References: None.

Co-investigators/Cooperators: D. Haymer, Univ. of Hawaii, Honolulu; H. Chang, ARS, Honolulu; APHIS S&T, Honolulu.

Work Plans for FY 92: Field evaluations of medfly genetic sexing strain will be completed. Other genetically selected strains, including the medfly "large" strain, and pupal color strains, as yet undeveloped Bactrocera species, await further study in future years.

III.C.1.c.0.1 Handler, A. CRIS 6615-22240-001-00D

Also: III.C.1.c.0.2

SY: 0.1

Objective: To identify and isolate dominant-acting male-sterility genes in B. melanogaster which result from neo-, anti-, or hypermorphic activity.

Significance: Mutant genes whose phenotype is due to either new (neo), antagonistic (anti) or overexpressive (hyper) activity are usually dominant to their wild type, or normal, allele. Such genes which result in male sterility may be used in other species to induce male sterility by, in effect, "poisoning" a process needed for fertility. Such genes could be used for sterilization in SIT or used to, more generally, control male reproduction.

Justification: Industry - III.H; Regulatory - III.XX, III.YY.

Constraints: Testing requires gene transformation.

References: None.

Co-investigators/Cooperators: D. Segal, Tel-Aviv Univ.

Work Plans for FY 92: None.

III.C.1.c.0.1 Handler, A. CRIS 6615-22240-001-00D

Also: III.C.1.c.0.2; III.C.1.d.0.1-0.2

SY: 0.1

Objective: To identify and isolate sex-determination genes in tephritid fruit flies.

Significance: In Drosophila, manipulation of sex-determination genes can result in two very effective ways of achieving male selection and sterilization. First, at least three major sex-determination genes are functional only in females due to sex-specific intron splicing. If other insects use a similar system, then the sex-specific splice sites could be dissected from the sex-determination gene and used to effect sex-specific expression of genes encoding selectable products (i.e., toxins or drug resistance). Secondly, one of the Drosophila sex-determination genes is necessary for female development and male fertility. A temperature-sensitive allele of this gene results in both chromosomal females and males both developing as sterile males, yielding the most efficient means of producing sterile males.

Justification: Industry - III.H; Regulatory - III.YY.

Constraints: Initial DNA hybridization screens for homologous sex-determination genes in nondrosophilid insects have not been successful. A lack of marked

(mutant alleles) and balancer (suppressors of recombination) chromosomes in Anastrepha make mutant screens difficult. More creative approaches using polymerase chain reaction technology and screens for functional domains may be required. Gene transformation would facilitate testing putative genes.

References: None.

Co-investigators/Cooperators: Potential: J. Belote, Syracuse Univ.; M. McKeown, Salk Institute.

Work Plans for FY 92: Will begin identifying highly conserved sequences among known sex-determination genes. Appropriate oligonucleotide primers from these sequences and sequences necessary for sex-specific RNA processing will be prepared for PCR studies.

0.2 Bioengineering

Also see McInnis III.C.1.c.0.1, Handler III.C.1.c.0.1, Handler III.C.1.c.0.1.

0.3 Strain Improvement

III.C.1.c.0.3 McInnis, D. CRIS 5320-22000-011-00D

SY: 0.1

Objective: Quality control of mass-produced tephritid fruit flies for sterile release or genetic control programs.

Significance: Improving the quality of mass-produced fruit flies for control programs will result in achieving the eradication of native fly populations sooner, at lower cost.

Justification: Regulatory - III.L, III.O, III.S.

Constraints: Development of low cost, highly predictive and reliable tests of fly quality in the field.

References: None.

Co-investigators/Cooperators: APHIS S&T, Honolulu; E. Jang, ARS, Hilo; R. Vargas, ARS, Honolulu.

Work Plans for FY 92: Collaborative work will continue to monitor the behavioral and genetic quality of flies for SIT programs by using small-scale free-releases,

field cage experiments, the newly developed sperm ID method to track field mating and isozyme variation.

d. Single Sex Strain

Also see McInnis III.C.3.d.

0.1 Mutation selection

Also see Handler III.C.1.c.0.1.

0.2 Bioengineering

III.C.1.d.0.2 Handler, A. *CRIS 6615-22240-001-08T*
USDA-CSRS grant
(expires 8/93)

Also: III.C.1.e

SY: 0.2

Objective: To develop new gene vectors by first testing, in insects, the mobility properties of the transposable elements Ac from maize, and Tcl from nematodes.

Significance: Most gene transformation vectors are derived from transposable elements which have inherent mobility properties. Some, such as the P-element, have species specific restrictions on the mobility. Studies on the Ac and Tcl elements indicate that they are considerably less restricted, and yet they share homologous functional domains with another gene vector from Drosophila, hobo. We are, therefore, encouraged to believe that they will be functional in insects and we will initially test them in Drosophila. If they are mobile in this insect, then they will be developed into a vector system and tested in other insects.

Justification: Industry - III.H; Regulatory - III.ZZ.

Constraints: Efficient gene transformation systems are available for almost all genetically well-defined organisms, including one insect species, Drosophila melanogaster. The lack of genetic information and tools in most other insects has been a major barrier to the development of transformation technology as it relates to DNA integration mechanisms as well as transformant selection. Differences in insect biology also affect possible means of introducing DNA into germ cells. Nevertheless, despite these limitations there is every reason to believe that gene transformation will be possible for all insects. Given the various problems that must be addressed, a major limitation is the

relatively few laboratories dedicated to this research. Efficient screening for transformants remains a barrier. Although mutant strains are available in some tephritid species, notably C. capitata, none of their wild type alleles have been cloned.

References: None.

Co-investigators/Cooperators: D. A. O'Brochta, Univ. of Maryland, College Park, MD.

Work Plans for FY 92: A mobility assay for the maize Ac transposon will be developed in collaboration with Dr. D. O'Brochta (Univ. of Maryland). Mobility properties of Ac in Drosophila and A. suspensa will be tested.

III.C.1.d.0.2 Handler, A. CRIS 6615-22240-001-08T
USDA-CSRS grant
(expires 8/93)

Also: III.C.1.e

SY: 0.1

Objective: To determine the restrictions on P gene vector mobility in nondrosophilids. Since a transposase cDNA shows at least limited function in its ability to catalyze P mobility, attention will be focused on putative co-factors from Drosophila, possible missing in other insects, which may be required to restore full mobility. Tests will initially be done using a transient embryonic mobility assay, and positive acting factors will be subsequently tested in germline transformation experiments.

Significance: The use of molecular biological techniques for biological control methods will require the ability to transfer DNA molecules, manipulated in vitro, into the genome of host insects. Due to practical, and possibly, socio-ecological constraints, the release of transformant insects for biological control may be limited in the short-term. However, use of transformant insects to genetically sex and sterilize males for sterile insect release may be more easily implemented than for other field applications. This is due to methods which allow laboratory containment of genetically altered breeding insects while limiting release to sterile-male progeny. Even if release of transformant insects is generally limited a vast amount of basic biological information will be gained as it relates to chemical resistance mechanisms, sex determination, hybrid sterility, and hormone action and metabolism which will be useful in

enhancing current insect management programs, and in developing new ones.

Justification: Regulatory - III.ZZ.

Constraints: Efficient gene transformation systems are available for almost all genetically well-defined organisms, including one insect species, Drosophila melanogaster. The lack of genetic information and tools in most other insects has been a major barrier to the development of transformation technology as it relates to DNA integration mechanisms as well as transformant selection. Differences in insect biology also affect possible means of introducing DNA into germ cells. Nevertheless, despite these limitations there is every reason to believe that gene transformation will be possible for all insects. Given the various problems that must be addressed, a major limitation is the relatively few laboratories dedicated to this research.

References:

O'Brochta, D. A. and A. M. Handler. 1988. Mobility of P elements in drosophilids and non drosophilids. Proc. Natl. Acad. Sci., USA 85:6052-6056.

Co-investigators/Cooperators: D. A. O'Brochta, Univ. of Maryland, College Park, MD. Potential: D. McInnis, ARS, Honolulu; D. Haymer, Univ. of Hawaii.

Work Plans for FY 92: Studies will be continued to test the P transposase cDNA function in A. suspense will be continued, especially conjunction experiments with Drosophila nuclear extracts and purified fractions.

III.C.1.d.0.2 Handler, A. CRIS 6615-22240-001-00D

Also: III.C.1.e

SY: 0.2

Objective: To develop efficient selection and screening systems for gene transformants in nondrosophilid insects.

Significance: Gene transformation technology will only be useful if efficient means are available to select for transformants. This is straightforward for Drosophila and other organisms in which mutant phenotypes can be reverted by the integration of the wild type gene for the mutant allele ("mutant rescue"). Such genes are used as marker DNA in the gene vector. Such markers are currently not available in nondrosophilid insects. A

dominant marker system (i.e. an integrated gene creating a new phenotype) is available which causes drug-resistance to a neomycin analog, however this system has worked inconsistently.

Justification: Industry - III.H; Regulatory - III.R, III.ZZ.

Constraints: Although mutant strains are available in some tephritid species, notably *C. capitata*, none of their wild type alleles have been cloned.

References: None.

Co-investigators/Cooperators: D. A. O'Brochta, Univ. of Maryland, College Park, MD.

Work Plans for FY 92: Gene-transfer selection will be tested using PCR analysis and putative markers including neomycin-resistance, β -galactosidase, luciferase, and β -glucuronidase.

Also see Handler III.C.1.c.0.1.

e. Gene Transfer in Wild Populations

Also see Handler III.C.1.d.0.2, Handler III.C.1.d.0.2, Handler III.C.1.d.0.2.

2. Behavioral Quality Control of Flies

Background - Quality control methods have been developed for medfly, Caribbean fruit fly and the Mexican fruit fly. Quality control manuals have been written for medfly that have been accepted by APHIS for large factories that furnish flies for eradication programs.

Needs - Techniques to select, during the rearing process, for better and more competitive flies are needed. Improvement of existing tests for species other than medfly are needed. Tests to determine the effectiveness of released flies in the field such as dispersal, mating and host finding need to be developed. A method to collect eggs in the field to determine the level of sterility is needed. Development of dyes and/or genetic markers would facilitate the identification of sterile flies in traps.

a. Laboratory Testing Methods

III.C.2.a Seo, S.

CRIS 5320-22000-001-00D

SY: 0.3

Objective: Determine and develop a model for the effects of temperature, rate of airflow and humidity on knockdown time, recovery from knockdown, flight ability, mating competitiveness and longevity of gamma-irradiated Mediterranean fruit flies.

Significance: The information would show the limits on handling sterile Mediterranean fruit flies effectively in sterile-insect technique programs and whether fluidization could be used to move the sterile fruit flies out of the release machines. The information may be used for development of a vehicle-mounted ground-release machine or aerial-release machine for the sterile fruit flies.

Justification: Industry - III.H; Regulatory - III.N, III.FF.

Constraints: Availability of Mediterranean fruit flies of proper age (without manipulation) for mating tests, laboratory space to hold and test the treated fruit flies, apparatus and funds.

References:

Brown and Associates. 1950. Unit Operations. John Wiley & Sons. New York. 611 PP.

Co-investigators/Cooperators: R. Vargas, ARS, Honolulu.
Potential: D. Pierce, APHIS Methods.

Work Plans for FY 92: Two methods of treatment will be used to study the effect of low temperatures on Mediterranean fruit flies. One method will treat the adults with little air movement. In the other method, air will flow through a load of fruit flies up to the fluidization rate. Temperatures will be about 2.2°C to 7.2°C. Flow-rate and relative humidity will be measured. Biological factors for study will be flight ability, mating propensity, longevity and sperm transfer.

III.C.2.a Seo, S.

CRIS 5320-43000-011-00D

SY: 0.3

Objective: Develop models for emergence or sterility, flight ability, mating competitiveness and longevity of gamma-irradiated males and females of the oriental fruit fly and melon fly. On completion of this work, research will be on shipping methods.

Significance: Models provide options for the field entomologist to plan and develop strategies for effective use of the sterilized fruit flies for eradication programs.

Justification: Industry - III.H; Regulatory - III.G, III.S, III.V, III.WW.

Constraints: Availability of yellow-strain oriental fruit fly and melon fly pupae for testing, laboratory space to hold treated fruit flies and funds.

References:

Steiner, L. F., W. C. Mitchell and A. H. Baumhover. 1962. Progress of fruit-fly control by irradiation sterilization in Hawaii and the Marianas Islands. *Int. J. Appl. Radiation and Isotopes* 13:427-434.

Co-investigators/Cooperators: R. Vargas, ARS, Honolulu.
Potential: APHIS S&T; University of Hawaii.

Work Plans for FY 92: Determine with respect to irradiation dose and various atmospheres the sterility, complete emergence data, mating competitiveness or propensity, flight ability and longevity of the fruit flies. The data will be correlated to dose. Oriental fruit fly will be studied first and work will be repeated with the melon fly. Then work will begin on shipping methods.

Also see Handler III.C.1.c.0.1, Calkins III.C.1.a.0.4.

b. Field Testing Methods

III.C.2.b Vargas, R. CRIS 5320-22000-013-00D

SY: 0.5 (Vargas), 0.5 (Spencer), 0.1 (Seo)

Objective: Develop field quality control methodology for Mediterranean fruit fly.

Significance: Very few tests are available to measure the behavior or effectiveness of sterile fruit flies in the field.

Justification: Industry - III.H; Regulatory - III.S.

Constraints: None.

References: None.

Co-investigators/Cooperators: J. Spencer, ARS, Kapaa; S. Seo and H. Chang, ARS, Honolulu; APHIS S&T, Hawaii Sterile Fruit Fly Facility.

Work Plans for FY 92: Conduct dispersal studies of sterile Mediterranean fruit flies in three different ecological habitats.

Also see Calkins III.C.1.a.0.4.

0.1 In Vitro Method for Determination of Sterility

III.C.2.b.0.1 Seo, S. CRIS 5320-43000-011-00D

SY: 0.1

Objective: Develop an in vitro method for determining the sterility of irradiated fruit flies.

Significance: An in vitro method would eliminate error in estimating sterility from nonmating and absence of irradiated sperm in the male.

Justification: Industry - III.G, III.H; Regulatory - III.S.

Constraints: Medium for the in vitro method.

References: None.

Co-investigators/Cooperators: Potential: E. Jang, ARS, Honolulu; F. Chang, University of Hawaii.

Work Plans for FY 92: Materials in the spermatheca or accessory gland will be analyzed if information is unavailable in scientific literature. Artificial mediums will be prepared according to the information and tested for sperm motility. Selected medium will be used for determining sterility in irradiated fruit flies.

0.2 Sperm ID for mating

III.C.2.b.0.2 McInnis, D. CRIS 5320-22000-011-00D

SY: 0.2

Objective: To monitor the field mating competitiveness of released sterile insects using a newly developed microscopic technique which can distinguish normal from irradiated sperm in mated females.

Significance: The ultimate success of an SIT program depends upon the ability of released males to find wild females in the field and mate with them. Monitoring this ability under actual field conditions through the recovery of trapped females, dissection and bioassay in the lab is now possible for the medfly and thus provides this crucial information to program officials.

Justification: Industry - III.H; Regulatory - III.VV.

Constraints: None.

References: None.

Co-investigators/Cooperators: J. Spencer and R. Vargas, ARS, Honolulu; APHIS S&T, Honolulu.

Work Plans for FY 92: Use the technique to evaluate and distinguish the field mating performance of several standard or genetically biased sex-ratio strains of medfly. Also, continue to monitor the ongoing SIT program in Kauai to assess the efficiency of released sterile males and females.

0.3 Dispersal

0.4 Mating

0.5 Host-finding

c. Identification of Species

0.1 Dyeing

Also see Moreno III.C.3.b.

0.2 Genetic markers

3. Irradiation, Marking, Packaging, Shipping and Release Techniques

Background - Sterilization, marking, shipping and release techniques have been established for fruit flies used in eradication programs.

Needs - Sterilizing rates have been set at rates much higher than necessary and as a result, the quality of flies have suffered. A rate that results in no threat to the SIT program yet results in high quality flies needs to be established. Irradiation in different atmospheres should be investigated for other species. Release

systems need to be improved especially in the area of non-chilled adult aerial releases. A combination of sterile releases and male annihilation techniques in the same program should be investigated.

a. Sterilization

III.C.3.a Moreno, D. S. CRIS 6204-43000-003-00D

SY: 0.2

Objective: Develop a method of sterilizing the Mexican fruit fly under controlled atmospheres to diminish damaging effects of irradiation seen in previous studies.

Significance: If the damaging effects of irradiation can be minimized or eliminated, the natural competitiveness of the male can be retained. This accomplishment would enhance the effectiveness of the SIT program already in place for the Mexican fruit fly.

Justification: Industry - III.G; Regulatory - III.O.

Constraints: Once a method is developed in the laboratory, the findings need to be confirmed in the field using feral insects. The collection of feral insects is seasonal and has to be done in Mexico. The collection of infested fruit can be from the native host, the yellow chapote, or from grapefruit in commercial orchards. The production of yellow chapote is short, whereas that of grapefruit is long. However with the latter, one would virtually have to buy the fruit to allow the infestations to occur. The competitive tests have to be done in Mexico. The closest location for us is about 100 miles south of the international border.

References: None.

Co-investigators/Cooperators: R. L. Mangan, ARS, Weslaco Texas; APHIS S&T, Mission, Texas. Potential: SARH, Centro del Instituto Nacional de Investigaciones Forestales y Agropecuarias (CINIFAP), General Teran, Nuevo Leon, Mexico.

Work Plans for FY 92: Irradiate pupae of the Mexican fruit fly under modified atmospheres (nitrogen, helium, carbon dioxide and low temperatures). Determine the fruit fly's survival, flight ability, competitiveness and sterility in the laboratory.

III.C.3.a Heath, R. CRIS 6615-22000-008-01R

SY: 0.1

Objective: Determine effect of radiation on pheromone production of irradiated medflies.

Significance: This research will provide information on the competitiveness of irradiated male fruit flies in attracting female flies.

Justification: Industry - III.G, III.H; Regulatory - III.N, III.O, III.V, III.XX.

Constraints: None.

References: None.

Co-investigators/Cooperators: P. Landolt, N. D. Epsky and J. Sivinski, ARS, Gainesville; APHIS S&T, Guatemala; E. Jang, ARS, Hilo, W. Enkerlin, Program Moscamed, Tapachula.

Work Plans for FY 92: Determine if the release rate and ratio of components released by irradiated male medflies is different than that observed from laboratory and wild male medflies using flies available in Guatemala. Depending on time available, this research will be conducted in a similar manner using flies available in Hawaii.

0.1 Irradiation

0.2 Atmospheres

0.3 Temperatures

0.4 Dosimetry

b. Dyeing

III.C.3.b Moreno, D. S. *CRIS 6204-43000-003-00D*

Also: III.C.2.c.0.1

SY: 0.3

Objective: Develop a backup internal marker for the Mexican fruit fly.

Significance: The development of an internal marker for the Mexican fruit fly would add security to the decision-process of calling a poor external-marked fly feral when in reality may be a sterile fly or vice versa. An

internal marker that also marks sperm would allow us to determine the efficacy of released males in inseminating feral females in their own habitat.

Justification: Industry - III.G; Regulatory - III.R.

Constraints: The screening of potential dyes (over 100 identified) in the laboratory and in field cages is a long process due to the long longevity (half life of 60 days) of the Mexican fruit fly. Most of the work in field cages has to be done in Mexico. Frequent overnight travel is necessary in order to set up tests and maintain insects.

References: None.

Co-investigators/Cooperators: R. L. Mangan, ARS, Weslaco, Texas. Potential: APHIS S&T, Mission, Texas.

Work Plans for FY 92: Screen potential markers already on hand. The screening process requires at least three weeks per marker. To check if the marker stains all the target tissues and determine persistence of stains in the tissues for three weeks from initial feeding. If the marker is promising and retesting is necessary, an additional five weeks is necessary. This is to narrow down dosage and determine mortality of insects in field cages; also, to determine if marked recaptured insects retain the stain through an additional week in the McPhail trap and another week in a preserving fluid. Successful candidates will finally be tested in large field-tree cages for three weeks after feeding. At this point all the work will be conducted in Mexico. Perhaps we will not be able to make more than two stains to the field the first year.

Also see Vargas III.C.3.d.

c. Packaging for Shipment

d. Release Systems

III.C.3.d Vargas, R.

CRIS 5320-43000-006-00D

Also: III.C.3.b

SY: 0.3 (Vargas), 0.1 (Seo)

Objective: Develop new SIT technologies (i.e. improved aerial release systems, automated ground release systems, new sterile fly marking systems, pupal release systems,

etc.) for cost effective eradication of Mediterranean fruit fly from Hawaii.

Significance: During the past year we have been involved in a large scale pilot test for eradication of Mediterranean fruit fly on the island of Kauai. New procedures would allow for enhancement of SIT component to eradication of Mediterranean fruit fly from Hawaii and be useful for more cost effective eradication of accidental introductions into the mainland U.S. by APHIS.

Justification: Industry - III.H; Regulatory - III.T, III.U, III.FF, III.SS, III.VV, III.WW.

Constraints: None.

References: None.

Co-investigators/Cooperators: J. Spencer, ARS, Kapaa; S. Seo, D. McInnis and H. Chang, ARS, Honolulu; APHIS S&T, Hawaii Sterile Fruit Fly Facility.

Work Plans for FY 92: Field release program of sterile flies to determine the degree of suppression of medfly populations in coffee fields.

III.C.3.d McInnis, D. CRIS 5320-22000-011-00D

Also: III.C.1.d; III.C.3.e

SY: 0.4

Objective: Evaluate the efficiency of single-sex sterile medfly releases in comparison to standard bisexual (males + females) releases.

Significance: Release of sterile fly populations involving all-males (98%) could improve the overall efficiency of sterile insect release programs against tephritid fruit flies, especially medflies.

Justification: Industry - III.H; Regulatory - III.W.

Constraints: Mass-production and release of several different kinds of sterile medfly populations, including all-males requires careful coordination to prevent strain contamination in rearing, and the availability of multiple, relatively homogeneous, field test sites that are sufficiently isolated from each other to avoid strain overlap in the field. Also, care must be taken to monitor internal breakdown of the genetic sexing strain and repurify it periodically from pure stock colonies.

References: None.

Co-investigators/Cooperators: J. Spencer, ARS, Kapaa; R. Vargas and H. Chang ARS, Honolulu.

Work Plans for FY 92: Final field evaluations of the pupal color medfly sexing strain will be completed in Hawaii leading to a decision on whether or not to recommend release of all-male strains to improve efficiency of SIT strains.

0.1 Pupal

0.2 Adult-Ground

0.3 Adult-Air

e. Combo Treatment - Sterile Female Released with MAT

Also see McInnis III.C.3.d.

4. Strategy and Tactics

Background - Overflooding ratios have been set arbitrarily in the past based on the availability of flies and rules of thumb. Monitoring of the ratios and distribution within the release areas are evaluated by trap catches only.

Needs - Although computer models have indicated the rate of eradication with different overflooding ratios, no one has ever determined this rate in the field. How the flies distribute themselves after they are released needs to be investigated to see if they indeed go to the same habitat as preferred by the wild flies. The monitoring traps need to be placed in these preferred sites.

III.C.4 Spencer, J.

CRIS 5320-22000-008-00D

SY: 0.1

Objective: Develop new and/or improved SIT technologies/methodologies for the eradication of Mediterranean fruit fly.

Significance: The evaluation of these new procedures should lead to the suppression of the medfly population on Kauai. Presumably, if these procedures work well on Kauai, they will be effective on the other Hawaiian islands.

Justification: Regulatory - III.H, III.O, III.P, III.S, III.T, III.U, III.W, III.FF, III.GG.

Constraints: Personnel and logistical problem associated with programs of this magnitude.

References: None.

Co-investigators/Cooperators: R. Vargas, S. Seo, D. McInnis and H. Chang, ARS, Honolulu; APHIS S&T, Hawaii Sterile Fruit Fly Facility.

Work Plans for FY 92: 1) Continue release of sterile medflies in McBryde coffee while increasing zone to include a "buffer" around coffee, 2) continue and complete comparison of aerial vs. ground release, 3) initiate studies in attempt to understand movement of released and wild med flies in host and non-host areas.

III.C.4 Seo, S.

CRIS 5320-22000-011-00D

SY: 0.5

Objective: Determine the optimum ratio of sterile fruit fly to normal wild fly eradication of Mediterranean fruit fly, Malaysian fruit fly, melon fly and oriental fruit fly.

Significance: Provide information for the field entomologist on planning strategies for sterile insect technique programs. Provide mass-rearing lab with information for producing realistic numbers of pupae for sterilization and use in sterile-insect technique programs.

Justification: Industry - III.H; Regulatory - III.WW.

Constraints: Seasonal availability of wild hosts of the Mediterranean fruit fly and, possibly, Malaysian fruit fly and funds. Wild fruit flies mature at rates that differ with the laboratory-strain.

References:

Seo, S. T., R. I. Vargas, J. E. Gilmore, R. S. Kurashima and M. S. Fujimoto. 1990. Sperm transfer in normal and gamma-irradiated laboratory-reared Mediterranean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 83(5):1950-1953.

Data on wild medflies and a cause for variability of sperm transfer to be published when latter work is completed.

Co-investigators/Cooperators: R. Vargas, ARS, Honolulu.

Work Plans for FY 92: Continue replicating mating ratio tests at sterile:wild fly ratios of 1:1 to 1:64 for medfly. Depending on change of priorities and host availability, data will be obtained in 2-3 years for Malaysian fruit fly, oriental fruit fly and melon fly, respectively.

III.C.4 Mangan, R.

CRIS 6204-43000-003

SY: 0.3

Objective: Enhance technology and design system for standardized field tests of Mexican fruit fly eradication methodology. Design trapping systems and fruit sampling systems to determine effectiveness of sterile fly dispersal, mating success, longevity and target population suppression for eradication programs in the Rio Grande Valley of Texas, southern California and northern states of Mexico.

Significance: Decisions for fruit fly eradication protocols such as the Mexican fruit fly protocol for the Rio Grande Valley include requirements of release rates for sterile males and rates of pest trapping. A vast amount of data from about 60 years of research on the ecology, behavior and bionomics of this species exists but has not been integrated to design a scientifically based method for determining the effects of sterile insect release on the target population during the generation when the pest is detected. Field tests of methods on natural populations of Mexican fruit flies can be designed to measure rates of sterility among native females and other effects of sterile fly release. Other information such as effects of combined bait spray and sterile fly release can be evaluated.

Justification: Industry - III.G, III.H, III.I;
Regulatory - II.B, III.B, III.P, III.S, III.T, III.U,
III.V, III.FF, III.MM, III.OO, III.SS, III.UU, III.WW,
III.XX.

Constraints: A method of directly estimating sterility rates of native populations is not available. Factors affecting trap effectiveness are poorly understood.

References: None.

Co-investigators/Cooperators: APHIS S&T; Mexican fruit producers; Robert Wharton, Texas A&M, College Station, TX.

Work Plans for FY 92: Determine infestation distributions of grapefruit and mangos from orchards with various degrees (mean larvae/fruit) of infestation. Analyze data from SIT releases in Baja California Sur. Test various oviposition traps for sampling reproductive capacity in chapotales in Nuevo Leon.

a. Distribution

b. Overflooding

c. Monitoring and Evaluation

D. Biocontrol

1. Pathogens

Background - No microbes have been identified as pathogens that occur in the field. Some work is being conducted in Austria on the toxic effects of ecto- and endotoxins of Bacillus thuringiensis. ARS scientists in Geneva, New York are exploring the possibility of using fungal toxicants as a control agent in bait sprays.

Needs - Work on the Bacillus thuringiensis toxins should continue and be applied in a field situation. Fungal toxicants as control agents in bait sprays may prove to be a suitable substitute for malathion. Other possible pathogens such as viruses, rickettsia, and bacteria should be screened for activity against the fruit flies that are in culture in the U.S.

a. Fungi

b. Bacteria

c. Virus

Also see Vail III.A.2.a.

d. Nematodes

III.D.1.d Lindegren, J. CRIS 5302-43000-018-00D

SY: 0.0

Objective: Evaluate residential soil drench treatments with the Kapow selection of Steinernema carpocapsae Weiser for the suppression of fruit fly larvae.

Significance: Medfly prepupal larval suppression has been evaluated under field conditions in Oahu and Kauai, Hawaii 29 different times at the selected 500 infective juveniles (IJ) concentration. Medfly mortality responses in Hawaii to the Kapow selection (Hawaii, Hawaii 1988 - average 3 tests in time; and Oahu, Hawaii 1990 - average 2 comparative tests - laboratory in vivo and commercial in vitro) were 97% and 99.6%, respectively. Since 1983 the following tephritid fruit flies have been evaluated in the field at the 500 IJ/cm² concentration on 3 different islands in Hawaii: Medfly -- Oahu, 1983, 1984, and 1990, Maui 1985, and Hawaii 1988; melon -- Hawaii 1988; oriental -- Hawaii 1988; caribbean -- Florida 1989; and walnut husk -- California 1989. The efficacious Kapow selection nematode, designed for insect pests with short windows of susceptibility, i.e., prepupal tephritid larvae is a commercially available, EPA exempt, biorational that would be quickly available as an additional tool for fruit fly control. This nematode needs to be evaluated in actual residential soil drench treatments for tephritid fruit fly suppression.

Justification: Industry - III.E; Regulatory - II.B, III.B, III.E, III.Z, III.FFF.

Constraints: Fiscal, no grant funds exist for this research. Collaborators located in tephritid fruit fly areas will be required if this dooryard nematode soil drench is to be evaluated under "real world" conditions.

References:

- Lindegren, J. E. and P. V. Vail. 1986. Susceptibility of Mediterranean fruit fly, melon fly and oriental fruit fly (Diptera: Tephritidae) to the entomophagous nematode, Steinernema feltiae, in laboratory tests. Environ. Entomol. 15:465-468.
- Lindegren, J. E., T. T. Wong and D. O. McInnis. 1990. Response of Mediterranean fruit fly, melon fly and oriental fruit fly (Diptera: Tephritidae) to the entomophagous nematode, Steinernema feltiae, in field tests in Hawaii. Environ. Entomol. 19:383-386.
- Lindegren, J. E. 1990. Field suppression of three fruit fly species (Diptera: Tephritidae) with Steinernema carpocapsae. Proc. and Abstr. V Internatl. Colloquim on Invertebr. Pathol. and Microbial Control, Adelaide, Australia.

Co-investigators/Cooperators: W. Schroeder, ARS, Orlando. Potential: R. Mangan, ARS, Weslaco; M. F. Purcell, ARS, Kapaa; APHIS S&T.

Work Plans for FY 92: None.

III.D.1.d Lindegren, J. CRIS 5302-43000-018-00D

SY: 0.0

Objective: Determine fruit fly adult susceptibility to Kapow selection Steinernema carpocapsae infective juveniles as they emerge through nematode infested soil.

Significance: Medfly adults were susceptible to entomopathogenic nematodes harbored in soil. Mortalities of medfly and oriental fruit fly adults emerging through a Kapow-vermiculite substrate were 22 and 24%, respectively, at 500 IJ/cm² (Hilo, Hawaii 1988). Medfly adult mortalities at the same concentration (preliminary evaluations, Kauai, Hawaii 1990) were 55% when emerging through a Kapow-soil substrate. Defining emerging adult fruit fly mortalities in various nematode-soil substrates could make the residential soil nematode drench applications more efficacious for augmentation of fruit fly suppression or eradication procedures.

Justification: Industry - III.E; Regulatory - II.B, III.B, III.E, III.Z, III.FFF.

Constraints: Development of ant predation free evaluations, fiscal, no grant funds exist for this research.

References:

Lindegren, J. E. and P. V. Vail. 1986. Susceptibility of Mediterranean fruit fly, melon fly and oriental fruit fly (Diptera: Tephritidae) to the entomogenous nematode, Steinernema feltiae, in laboratory tests. Environ. Entomol. 15:465-468.

Co-investigators/Cooperators: M. C. Purcell, ARS, Kapaa; R. Vargas, ARS, Honolulu. Potential: APHIS S&T.

Work Plans for FY 92: None.

III.D.1.d Lindegren, J. CRIS 5302-43000-018-00D

SY: 0.2

Objective: Evaluate large scale field fruitfly suppression with commercially produced Kapow selection (Steinernema carpocapsae Mexican strain) nematodes at a lower, more economical concentration.

Significance: Though decimated by ant predation, small scale field evaluation in Oahu, Hawaii (1990) indicated the infective juveniles (IJ) of Kapow selection would be efficacious at a 10-fold reduction in IJ concentrations as used in previous tests. Combined mortalities (commercial and laboratory produced Kapow) listed consecutively for days 0, 2 and 5 post applications were: 79.5, 35.4, and 38.9 at 15 IJ/cm²; 90.4, 71.0, and 22.6

at 50 IJ/cm²; 99.5, 76.4, and 5.3 at 150 IJ/cm²; and 99.6, 96.8, and 18.0 at 500 IJ/cm². Definitive large scale medfly suppression field trials should be conducted with Kapow IJ concentrations of 50/cm² in Kauai coffee plantings. An additional test should be conducted with the Mexican fruit fly in Mexico citrus plantings at the Texas border. The naturally occurring predator populations, i.e. ants, should be monitored for deleterious effects. The persistence and possible establishment of entomopathogenic nematodes in a continuous fruitfly host producing habitat, i.e. coffee plantings, should also be evaluated.

Justification: Industry - III.E; Regulatory - II.B, III.B, III.E, III.Z, III.FFF.

Constraints: Purchase of Kapow nematodes at \$.50/10⁶ from Bioenterprises Pty. Ltd., Australia. Collaborators on Kauai, Texas and Mexico will be required. Isolated coffee or citrus plantings will be necessary for fruitfly adult population comparisons. Also the availability of wild medfly or Mexican fruit fly hosts, results of initial Mexican fruit fly susceptibility tests and fiscal support.

References:

- Lindegren, J. E. and P. V. Vail. 1986. Susceptibility of Mediterranean fruit fly, melon fly and oriental fruit fly (Diptera: Tephritidae) to the entomogenous nematode, Steinernema feltiae, in laboratory tests. Environ. Entomol. 15:465-468.
- Lindegren, J. E., T. T. Wong and D. O. McInnis. 1990. Response of Mediterranean fruit fly, melon fly and oriental fruit fly (Diptera: Tephritidae) to the entomogenous nematode, Steinernema feltiae, in field tests in Hawaii. Environ. Entomol. 19:383-386.
- Lindegren, J. E. 1990. Field suppression of three fruit fly species (Diptera: Tephritidae) with Steinernema carpocapsae. Proc. and Abstr. V Internatl. Colloquim on Invertebr. Pathol. and Microbial Control, Adelaide, Australia.

Co-investigators/Cooperators: R. Mangan, ARS, Weslaco; R. Vargas, ARS, Honolulu. Potential: R. Messing, Univ. of Hawaii, Honolulu; M. C. Purcell, ARS, Kapaa; APHIS S&T.

Work Plans for FY 92: Conduct large scale medfly suppression field trial in Kauai coffee and Mexico citrus plantings with Kapow selection S. carpocapsae at the 50 IJs/cm² concentration.

III.D.1.d Lindegren, J. CRIS 5302-43000-018-00D

Also: III.A.2.a

SY: 0.2

Objective: Determine if osmotically desiccated nematodes can be utilized with a fruit fly bait for adult control.

Significance: Active infective juveniles (IJ) were regurgitated by medfly adults (Hilo, Hawaii 1988) when ingested in low osmotic pressure bait formulations. Osmotically desiccated inactive IJs in high osmotic bait formulations were ingested and retained by medfly adults in limited evaluations (Kauai, Hawaii 1990). If effective, nematodes delivered as a bait would offer an economical biological adulticide for application to areas where conventional spray applications are not allowed.

Justification: Industry - III.E; Regulatory - II.B, III.B, III.E, III.Z, III.FFF.

Constraints: Bait-nematode formulations would require laboratory and field evaluation.

References: None.

Patent: Data submitted for patent application.

Co-investigators/Cooperators: C. O. Calkins, ARS, Gainesville; M. C. Purcell, ARS, Kapaa. **Potential:** APHIS S&T.

Work Plans for FY 92: Evaluate Kapow selection IJ response to candidate medfly baits.

2. Parasitoids

Background - Pilot tests in Hawaii and Florida are currently testing the feasibility of inundative releases of two different species of parasites for control of fruit flies in the field.

Needs - The pilot program in Hawaii is nearing completion. Application will go into effect in the medfly eradication program on Kauia as a means of lowering the population before and/or during the release of sterile flies. The pilot test in Florida has another year to go before recommendations can be made. Use of other parasite species in these types of programs is limited by the lack of rearing techniques. Species used in release studies were those that were easily reared and not necessarily the most effective parasite. The effectiveness of all of these parasite species needs to be determined. No means is available to determine the adult population of parasites. No attractant or trap is available. The most abundant parasite species varies

from year to year. The weather patterns and other factors that affect parasite numbers from year to year are unknown.

a. Exploration and Introduction

III.D.2.a Sivinski, J. CRIS 6615-22000-009-00D

SY: 0.1

Objective: To discover and introduce parasites of fruit flies.

Significance: If parasites can be established which significantly lower the population of fruit flies, less damage to fruit will occur, and it will be easier and less expensive to employ control measures such as sterile fly or inundative parasite releases. There is even a possibility of lowering infestation rates below economically significant levels.

Justification: Regulatory - III.K.

Constraints: Many of the "cheaply" obtained parasite species have been introduced. These were largely discovered decades ago in explorations for Bactrocera spp. parasites and established in Hawaii. Further exploration, particularly in Africa and Latin America, will call for long term field work (at least 1-2 years) and overseas collaborators.

References: None.

Co-investigators/Cooperators: R. Wharton, Texas A&M Univ.; R. Baranowski, Univ. of Florida.

Work Plans for FY 92: No explorations are planned for 1992.

b. Rearing and Handling

III.D.2.b Schroeder, W. CRIS 6617-22000-007-00D

Also: III.D.2.c; III.D.2.c.0.4

SY: 0.2

Objective: Development of tephritid parasite rearing for use in population suppression. Use the Caribbean fruit fly as the host. Evaluate and develop techniques for rearing native and exotic parasites for mass rearing and release.

Significance: The parasites can be utilized as an alternative to aerial bait sprays in conjunction with the sterile insect technique program.

Justification: Industry - III.C, III.E, III.H;
Regulatory - III.B, III.J, III.K.

Constraints: None.

References: None.

Co-investigators/Cooperators: C. Calkins and J. Sivinski, ARS, Gainesville; T. Wong, ARS, Honolulu; R. Baranowski, Gainesville, University of Florida.

Work Plans for FY 92: Review work in progress and extend research to include other fruit fly parasites currently not produced in mass culture. Many native parasites have not been reared in the laboratory, their life cycle is poorly defined and little basic information is available. Work will be limited to collecting, then rearing the parasites to obtain this basic information. The parasite will then be evaluated for laboratory rearing.

III.D.2.b Wong, T.

CRIS 5320-24000-005-00D

Also: III.D.2.b.0.1

SY: 0.5

Objective: Development of techniques to mass rear braconid fruit fly parasitoids for use in field releases to suppress fruit flies.

Significance: Augmentative releases of laboratory-reared parasitoids would provide an adjunct methodology to suppress fruit flies.

Justification: Regulatory - III.B, III.J.

Constraints: Environmental concerns such as the potential effects of the parasitoid releases on nontarget species as well as their natural enemies on release and surrounding areas.

References:

- Wong, T. T. Y., M. M. Ramadan, D. O. McInnis and N. Mochizuki. 1990.
Influence of cohort age and host age on oviposition activity and offspring sex ratio of Biosteres tryoni (Hymenoptera: Braconidae), a larval parasitoid of Ceratitis capitata (Diptera: Tephritidae). J. Econ. Entomol 83:779-783.

Wong, T. T. Y. and M. M. Ranadan. Mass rearing biology of larval parasitoids (Hymenoptera: Braconidae: Opiinae) of tephritid flies (Diptera: Tephritidae) in Hawaii. Book chapter in Advances in Insect Rearing and Pest Management, edited by T. E. Anderson and N. C. Leppla. (Accepted 12/90).

Co-investigators/Cooperators: H. Chang, D. McInnis, E. Harris and R. Vargas, ARS, Honolulu; M. Purcell, ARS, Kapaa; F. Chang, Univ. of Hawaii, Honolulu. Potential: E. Jang, ARS, Hilo.

Work Plans for FY 92: Production of ca. 1 million Diachasmimorpha longicaudata and 200,000 Psytalia fletcheri per week in the laboratory. From January to the end of 1992, B. longicaudata will be released in four 1-mile² plots at the rate of 5 female parasitoids to every female oriental fruit fly. Trap catches and fruit infestations from these 4 release plots will be compared with 4 equal-size untreated plots. P. fletcheri will be released in the Moloaa area and the number of parasitoids released per week will be 5 female parasitoids to 1 female melon fly.

0.1 Quality control

III.D.2.b.0.1 Calkins, C. O. CRIS 0500-00001-018-00D
(Funds will terminate
FY 94)

Also: III.D.2.e

SY: 0.2

Objective: To determine the effectiveness of parasites on the Caribbean fruit fly presently found in Florida.

Significance: Several parasites are presently found in Florida that attack Caribbean fruit fly larvae and pupae. The significance of these parasites is unknown since, rearing procedures have not been developed, and inundative or introductive releases have not been attempted. Mating and foraging behaviors have not been studied to determine their efficiency in specific habitats.

Justification: Regulatory - III.B, III.J, III.K.

Constraints: Inadequate knowledge of the biology of each species.

References: None.

Co-investigators/Cooperators: W. Schroeder, ARS, Orlando; J. Sivinski, ARS, Gainesville.

Work Plans for FY 92: To collect species from the field and to develop rearing technology for those species.

Also see Wong III.D.2.b.

c. Inundative Releases

III.D.2.c Purcell, M. F. CRIS 5320-24000-004-00D

SY: 0.5 (Purcell), 0.5 (Wong), 0.1 (Vargas)

Objective: Determine the potential of parasites of the melon fly and the oriental fruit fly for reducing populations of fruit flies as part of the overall strategy of eliminating fruit flies in Kauai.

Significance: As public concern about the adverse effects of insecticides on the environment mounts, it is becoming increasingly difficult to use a chemical insecticide approach in area-wide management and eradication programs. Biological control has the advantage of having minimal adverse effects on the environment, being highly specific to the target organism and thus would most likely gain ready acceptance by the public.

Justification: Regulatory - III.B, III.J.

Constraints: Quantitative estimates of the existing population of melon and oriental fruit flies is lacking. Therefore, it is difficult to assess the number of parasites that will be required to adequately suppress populations of fruit flies. Limitations of space for rearing fruit fly parasites may affect the scope of the program.

References: None.

Co-investigators/Cooperators: T. Wong and R. Vargas, ARS, Honolulu; R. Messing, Univ. of Hawaii, Kauai.

Work Plans for FY 92: Evaluate the efficacy of augmentative releases of parasitoids of reducing populations of oriental fruit fly in guava and melon fly in vegetable commodities (Team effort: Tim Wong (USDA, Honolulu), Russell Messing (Univ. Hawaii)).

Also see Schroeder III.D.2.b.

0.1 Mediterranean fruit fly

0.2 Melon fly

III.D.2.c.0.2 Wong, T.

CRIS 0500-00001-029-00D
(Pilot Test)

Also: III.D.2.c.0.3; IV.A.7.b

SY: 0.5 (Wong), 0.5 (Purcell), 0.5 (Spencer)

Objective: To determine the feasibility of integrating augmentative releases of parasitoids with the sterile insect technique (SIT) to suppress and/or eradicate natural populations of the oriental fruit fly and the melon fly.

Significance: Development of this integrated method will provide a critical tool for suppression/eradication of two major quarantine pests with an economic impact measured in millions of dollars in the event of their establishment on the U. S. mainland. This integrated method will provide an important tool for use in the Hawaiian tri-fly program if and when the program is implemented. Moreover, this integrated method does not require the use of insecticides and might be more effective, less costly, and much more ecologically acceptable.

Justification: Regulatory - III.B, III.J.

Constraints: No native plants or endangered plant or animal species are known to occur in the test area and consequently the test represents no threat to such species. However, environmental studies will be carried out to evaluate any potential effect of parasitoid releases on nontarget species.

References:

Wong, T. T. Y, M. M. Ramadan, D. O. McInnis, N. Mochizuki, J. I. Nishimoto and J. C. Herr. Augmentative releases of Diachasmimorpha tryoni (Hymenoptera: Braconidae) to suppress Mediterranean fruit fly (Diptera: Tephritidae) population in Kula, Maui, Hawaii. J. Biological Control. (Accepted September 28, 1990.)

Co-investigators/Cooperators: M. Purcell, ARS, Kauai; R. Vargas, D. McInnis and E. Harris, ARS, Honolulu; R. Messing, University of Hawaii, Kauai. Potential: APHIS S&T.

Work Plans for FY 92: Production of ca. 1 million Diachasmimorpha longicaudata and 200,000 Psytalia fletcheri per week in the laboratory. From January to the end of 1992, B. longicaudata will be released in four 1-mile² plots at the rate of 5 female parasitoids to every female oriental fruit fly. Trap catches and fruit

infestations from these 4 release plots will be compared with 4 equal-size untreated plots. P. fletcheri will be released in the Molooa area and the number of parasitoids released per week will be 5 female parasitoids to 1 female melon fly.

0.3 Oriental fruit fly

Also see Wong III.D.2.c.0.2.

0.4 Anastrepha

III.D.2.c.0.4 Sivinski, J. CRIS 0500-00001-018-00D
(terminates FY-93)

Also: III.D.2.e; IV.A.7.b

SY: 0.3

Objective: Development of techniques to control fruit flies with inundatively released parasites.

Significance: Use of inundative parasite releases could be a nonchemical means of suppressing numbers of flies in high population density areas that serve as foci of infestation. Parasites might also be used in conjunction with sterile males to more effectively eradicate pest populations.

Justification: Regulatory - III.J.

Constraints: A pilot test in cooperation with the University of Florida and the Florida Division of Plant Industry is now in its early stages. Florida may not have pass-through funds from APHIS to continue the program at the same level.

References:

- Sivinski, J. and C. O. Calkins. 1991. Sexually dimorphic developmental rates in the Caribbean fruit fly Anastrepha suspensa (Loew). Environ. Entomol. 19:1491-1495.
- Sivinski, J. and B. Smittle. Effects of gamma radiation on the development of the Caribbean fruit fly (Anastrepha suspensa) and the subsequent development of its parasite Deschasmumorphia longaudata. Entomol. Exp. Et. Appl. (In press, accepted 1/90).
- Sivinski, J. 1991. Effects of host fruit morphology on parasites of the Caribbean fruit fly by Deschasmumorphia longaudata. Entomophaga. (In press, accepted 6/90).

Co-investigators/Cooperators: R. Baranowski, Univ. of Florida; C. Calkins, ARS, Gainesville; D. Harris, Florida Division of Plant Industry.

Work Plans for FY 92: Releases of parasites and a combination of parasites and flies will be carried out during 1992. Techniques to separate female fly larvae for parasite hosts will be tested on a large scale.

III.D.2.c.0.4 Calkins, C. O. CRIS 0500-00001-018-00D
Funds will terminate
FY-94.

Also: III.D.2.d

SY: 0.2

Objective: Development of a system for Caribbean fruit fly population suppression with the use of inundative releases of parasites and sterile flies.

Significance: The use of these techniques to suppress fly populations in urban areas would solve the problem of chemical sprays for removing the threat that these populations would pose to adjacent fly-free areas.

Justification: Regulatory II.B, III.B, III.J.

Constraints: A pilot test is presently underway in south Florida to determine if the use of parasites and sterile flies or of parasites alone will suppress populations of flies in urban areas. The Florida Division of Plant Industry may not receive the normal allotment of pass-through funds from APHIS in FY 92 to continue the strong support necessary to complete the study.

References: None.

Co-investigators/Cooperators: J. Sivinski, ARS, Gainesville; Gaskalla, Harris, and Burns, Florida Division of Plant Industry, Gainesville; R. Baranowski, Univ. of Florida; APHIS S&T, Gainesville.

Work Plans for FY 92: Release inundative level of parasites and/or sterile flies and monitor effects on the native population.

Also see Schroeder III.D.2.b.

d. Combination Systems

Also see Calkins III.D.2.c.0.4.

e. Behavior

Also see Calkins III.D.2.b.0.1, Sivinski III.D.2.c.0.4.

0.1 Semiochemicals

Also see Jang I.H.

0.2 Acoustics

0.3 Ecology habitat

III.D.2.e.0.3 Harris, E. CRIS 5320-24000-005-00D

SY: 0.25

Objective: Biology and ecology of Biosteres arisanus parasitoids in urban areas of Hawaii.

Significance: A better understanding of the biology and ecology of B. arisanus would enable us to understand why B. arisanus is the most effective and widely distributed parasitoid in Hawaii. Ecological information could provide clues as to what can be done to make the larval parasitoids more effective and widely distributed and indicate when, where and under what conditions to use inoculative and inundative releases of Biosteres parasitoids.

Justification: Industry - III.F; Regulatory - III.B, III.J, III.K.

Constraints: The knowledge base about the biology and ecology of parasitoids in the field is very limited particularly their mating behavior. More basic information is needed to maximize the use of augmentation release technology for parasitoids and interpret correctly the results.

References:

- Harris, E. J. and R. Y. Okamoto. 1983. Description and evaluation of a simple method for collection of the parasite Biosteres oophilus (Hymen.: Braconidae). Entomophaga 28:241-243.
- Harris, E. J., R. Y. Okamoto, C. Y. L. Lee and T. Nishida. Suitability of Dacus dorsalis and Ceratitis capitata (Diptera: Tephritidae) as hosts of Biosteres arisanus (Sonana) Entomophaga. (In press).

Co-investigators/Cooperators: R. Vargas, ARS, Honolulu; N. Tanaka, California Hawaii Fruit Fly Laboratory, Honolulu City and County Board of Water Supply.

Potential: T. Wong, ARS, Honolulu; M. Purcell, ARS, Kauai; Hawaii Dept. of Agriculture; Univ. of Hawaii.

Work Plans for FY 92: Experimental plots will be set up in Honolulu in residential and urban areas to observe fruit fly and parasitoid behavior in the field. Fruit

fly and parasitoid populations will be monitored with traps and with fruit collections. Oviposition and mating behavior will be studied in detail in outdoor cages over host plants.

3. Predators

IV. Fundamental Biology

A. Ecology and Behavior

Background - Dispersal studies have been conducted with medflies and Caribbean fruit flies to determine the effects of radiation doses on movement and longevity in the field. Mating behavior studies with medflies and caribflies were conducted in connection with quality evaluations. The use of acoustics in the mating repertoire was studied in detail for these 2 species.

Needs - Dispersal rates and movement studies have not been conducted on several economic fruit fly species. Detailed studies of mating behavior results in insight into the factors that influence calling behavior, attraction to traps, aggregation sites, etc. Diurnal rhythm studies have led to proper timing of mating tests, insights as to when flies are present in certain habitats, and when and where to place attractants and traps to observe and rate the effectiveness of traps and attractants. Development of population spatial models will lead to the development of a geographic information system (GIS) that will help predict areas where certain flies will be able to overwinter or where they might be a seasonal problem.

1. Movement

a. Dispersal

IV.A.1.a Harris, E.

CRIS 5320-24000-005-00D

Also: IV.A.1.b; IV.A.7

SY: 0.25

Objective: Development of a stratified sampling system for monitoring B. cucurbitae and B. latifrons and their host plants in urban areas.

Significance: With the development of this system it would be possible to obtain better knowledge about dispersal behavior, habitat, and host preferences of both species simultaneously since the two species may share the same host plants and habitats. A system is required which includes more variables for appraising habitat changes between seasons and years.

Justification: Industry - I.A; Regulatory - I.A, I.B. I.H.

Constraints: The knowledge base about the biology and ecology of female melon flies and Malaysian fruit flies in the field is very limited. A better monitoring system is needed which has a component in it to sample male and female flies as well as fruit maturity and phenology of the host plants. This information is needed to evaluate male annihilation technology for these species and to maximize the effectiveness of sterile insect release technology in Hawaii and the mainland to suppress or eradicate melon and Malaysian fruit flies.

References:

- Harris, E. J., J. M. Takara and T. Nishida. 1986. Distribution of the melon fly, Dacus cucurbitae, (Diptera: Tephritidae) and host plants on Kauai. Environ. Entomol. 15:488-493.
- Harris, E. J. and C. Y. L. Lee. 1989. Influence of bittermelon, Momordica charantia L. (Cucurbitae) on melon fly, Dacus cucurbitae Coquillett, (Diptera: Tephritidae) distribution on the island of Molokai, Hawaii. Proc. Hawaii Entomol. Soc. 29:49-56.
- Harris, E. J., C. Ford-Livene and C. Y. L. Lee. Population monitoring of tephritidae fruit flies by stratified sampling on the island of Oahu, Hawaii. In First International Symposium on Fruit Flies in the Tropics, March 10-16, 1988. (In press).

Co-investigators/Cooperators: N. Liquido, ARS, Hilo; R. Cunningham, ARS, Honolulu; N. Tanaka, California Hawaii Fruit Fly Laboratory, Honolulu City and County Board of Water Supply. Potential: Hawaii Dept. of Agriculture; University of Hawaii.

Work Plans for FY 92: Experimental plots will be set up in community gardens, residential areas and on farms to monitor both species of fruit flies. Male lure and liquid lure traps will be used to monitor adult activity. Fruit collections will be made to identify infested hosts, host preference of the females and infestation rates. The distribution of the Malaysian fruit fly and the melon fly will be plotted and the host plants they share identified.

IV.A.1.a Harris, E.

CRIS 5320-24000-005-00D

Also: IV.A.1.b; IV.A.3.a; IV.A.7

SY: 0.25

Objective: Survey of the Malaysian fruit fly, Bactrocera latifrons on the islands of Oahu and Kauai.

Significance: The Malaysian fruit fly was introduced into Hawaii in 1983. This is the fourth economically important tephritid fruit fly present in Hawaii and the most interesting regarding its ecology and habits. In

planning a strategy for eradication of all tephritid fruit fly species from Hawaii, logically the last species introduced may be the least established and easier to eradicate. The Malaysian fruit fly was reported to be present only on the island of Oahu. A survey is needed on all the islands in Hawaii to determine the distribution, abundance, and host preference of the Malaysian fruit fly. The survey will provide basic ecological information necessary to eradicate this species from Hawaii.

Justification: The biotic potential of this species needs to be determined in relation to its ability to spread and invade new areas in the continental U. S.

Constraints: Basic information needs to be developed on the ecological niches used by the Malaysian fruit fly and host plants attacked only by this fly and the host plants shared with other tephritid fruit flies. Also, there is a need to identify the parasitoids in Hawaii which are effective in parasitizing B. latifrons. These parasitoid species may be effective candidates for inoculative and inundative releases alone or in combination with sterile B. latifrons releases.

References:

- Vargas, R. I. and T. Nishida. 1985. Life history and demographic parameters of Dacus latifrons (Diptera: Tephritidae). J. Econ. Entomol. 78:1242-1244.
- Vargas, R. I. and T. Nishida. 1985. Survey for Dacus latifrons (Diptera: Tephritidae). J. Econ. Entomol. 78:1311-1314.

Co-investigators/Cooperators: N. Liquido, ARS, Hilo; N. Tanaka, California Hawaii Fruit Fly Laboratory.
Potential: R. Vargas, ARS, Honolulu; Hawaii Dept. of Agriculture; Univ. of Hawaii.

Work Plans for FY 92: A survey is being conducted in Honolulu county using male lure and liquid lure traps to monitor the population in community gardens, around home sites and on farms. In all these locations, solanaceous and other fruits are collected to identify host plants of the Malaysian fruit fly. The rate of colonization is indicated by the discovery of new hosts not reported previously.

IV.A.1.a Harris, E.

CRIS 5320-43000-008-00D

Also: IV.A.1.b; IV.A.3.a; IV.A.7

SY: 0.25

Objective: Biology and ecology of the Mediterranean fruit fly in Hawaii.

Significance: Among the gaps in the knowledge about the biology and ecology of the Mediterranean fruit fly is dispersal patterns and oviposition behavior of females in colonizing widely distributed host fruits such as strawberry guava and mock orange in urban areas.

Justification: Industry - I.A; Regulatory - I.A, I.B, I.H.

Constraints: None.

References:

- Harris, E. J, and C. Y. L. Lee. 1987. Seasonal and annual distribution of the Mediterranean fruit fly (Diptera: Tephritidae) in Honolulu and suburban areas of Oahu, Hawaii. Environ. Entomol 16:1273-1282.
- Harris, E. J. and C. Y. L. Lee. 1989. Mediterranean fruit fly, Ceratitidis capitata (Diptera: Tephritidae) development in coffee in wet and dry habitats. Environ. Entomol. 18:1042-1049.

Co-investigators/Cooperators: R. Vargas and R. Cunningham, ARS, Honolulu; N. Tanaka, California Hawaii Fruit Fly Laboratory; Honolulu City and County Board of Water Supply. Potential: T. Wong, ARS, Honolulu; Hawaii Dept. of Agriculture.

Work Plans for FY 92: None for FY 92. Will begin in FY 93.

IV.A.1.a *Liquido, N. J.* CRIS 5320-22000-012-00D
5320-22000-013-00D

SY: 0.1

Objective: Quantification of the dispersal characteristics of the adults of the Mediterranean fruit fly, oriental fruit fly, melon fly, and Malaysian fruit fly.

Significance: One of the factors influencing effective implementation of the sterile insect technology is the spacing and pattern of release points, lines or grids. This research will provide this extremely important information for the oriental fruit fly, melon fly, and Malaysian fruit fly and will compliment the data which the Tropical Fruit and Vegetable Research Laboratory has gathered for the Mediterranean fruit fly. Furthermore, results on the dispersal will improve the present method of predicting the extent of infestation of the accidentally introduced, invading fruit fly population in

the mainland U. S. Furthermore, dispersal data will be useful in developing sampling protocols for established fruit fly populations in their natural habitats.

Justification: Industry - III.G, III.H; Regulatory - I.M., III.P, III.T, III.U.

Constraints: None.

References: None.

Co-investigators/Cooperators: R. Plant, Univ. of California, Davis; APHIS S&T; CDFA.

Work Plans for FY 92: Quantify movement of medfly in a habitat with fruiting hosts using trimedlure and ceralure "baited" traps; perform preliminary work on movement of B. latifrons.

b. Host-finding

Also see Harris IV.A.1.a, Harris IV.A.1.a, Harris IV.A.1.a.

2. Mating

IV.A.2 *Sivinski, J.*

CRIS 6615-22000-009-00D
6615-43000-006-00D

SY: 0.3

Objective: Understand the role of sexual selection in the evolution of the fruit fly reproductive behavior.

Significance: Sterile-male releases depend on the sexual competitiveness of mass-reared insects to effect control. A thorough understanding of male behaviors and female choice helps determine realistic quality control criteria. A knowledge of lek formation and where it occurs can lead to the best placement of traps or aid other control measures.

Justification: Regulatory - III.S, III.T.

Constraints: Parts of such a study requires substantial field work. At present, the time investment is unrealistic and a collaborator or assistant located near wild fly populations has not been located. Funds to pay for an assistant have not been obtained.

References:

- Sivinski, J. M. 1984. Effects of sexual experience on male mating success in a lek forming tephritid Anastrepha suspensa (Loew). Fla. Entomol. 67(1):126-130.
- Webb, J. C., J. M. Sivinski and C. Litzkow. 1984. Acoustical behavior and sexual success in the Caribbean fruit fly, Anastrepha suspensa (Loew). Environ. Entomol. 13(3):650-656.
- Sivinski, J. M., T. Burk and J. C. Webb. 1984. Acoustic courtship signals in the Caribbean fruit fly, Anastrepha suspensa (Loew). Anim. Behav. 32(4):1011-1016.
- Sivinski, J. M. and J. C. Webb. 1986. Changes in a Caribbean fruit fly acoustic signal with social situation (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 79:146-149.
- Sivinski, J. M. and B. Smittle. 1987. Male transfer of materials in the Caribbean fruit fly, Anastrepha suspensa (Loew) (Diptera: Tephritidae). Fla. Entomol. 70:233-238.
- Webb, J. C., J. M. Sivinski and B. Smittle. 1987. Acoustical courtship signals and sexual success in irradiated Caribbean fruit flies (Anastrepha suspensa (Loew)) (Diptera: Tephritidae). Fla. Entomol. 70:103-109.
- Sivinski, J. M. 1988. Lekking and the small scale distribution of the sexes in the Caribbean fruit fly (Anastrepha suspensa (Loew)). J. Insect Behav. 2:3-13.

Collaborators: R. Mankin, ARS, Gainesville.

Work Plans for FY 92: Publications on the significance of male size to female mate choice and the effect of mating on female longevity will be prepared/published.

IV.A.2 Mankin, R.

CRIS 6615-43000-006-00D

SY: 0.6

Objective: Increase knowledge about fruit fly acoustical communication that can be used for insect control. The initial emphasis of this research is on mate selection processes, integration of sound with chemical and visual stimuli and comparison of communication in closely related species.

Significance: A better understanding of communication processes is necessary to determine whether acoustical methods can be devised for fruit fly control. Mate selection is more complicated in fruit flies than in Lepidoptera, where research on semiochemicals led to development of sex pheromone applications. Previous studies of Drosophila, Anastrepha and other fruit flies have demonstrated that acoustical stimuli play a significant role in the mating process. However, it is not yet possible to monitor fruit fly populations or control fruit fly mating by use of acoustical stimuli. Some success has occurred in determining whether fruit fly larvae are present in individual fruit. Also, closely related species have been distinguished by identifying differences among their calling and courtship songs.

Justification: Industry - II.G; Regulatory - III.B, III.PP.

Constraints: None.

References: None.

Co-investigators/Cooperators: J. Sivinski, ARS, Gainesville; A. Malavasi, Univ. of Sao Paulo, Brazil; T. Forrest, National Center for Physical Acoustics, Oxford. Potential: T. Walker, Univ. of Florida, Gainesville; D. Robacker, ARS, Weslaco.

Work Plans for FY 92: Develop and test a digital signal processing program that analyzes and discriminates among acoustical signals produced by different fruit fly species.

Also see Landolt I.H.

3. Egg-Laying

a. Host Selection

IV.A.3.a Mangan, R.

CRIS 6204-43000-003

Also: IV.A.7

SY: 0.2

Objective: Determine the rate that fruit flies switch hosts between native plants such as yellow chapote and Spanish plum and commercial citrus and mango. Since the hosts do not fruit at the same time, this can be estimated by comparing the age structures of populations sampled in the hosts habitats at the same time and determining the degree of overlap in age structure. This research will focus on determining most efficient application of suppression technology.

Significance: If most flies captured in commercial hosts arrive from native host habitats and are already mated, there is little gain in treating the commercial orchards with sterile insects. If the populations are distinct and do not switch hosts, suppression efforts in native fruit habitats will not reduce commercial pest problems.

Justification: Industry - III.A, III.I; Regulatory - III.P, III.T, III.OO, III.UU, III.DDD, III.FFF.

Constraints: None.

References: None.

Co-investigators/Cooperators: Mexican fruit producers.

Work Plans for FY 92: Determine phenology of infestation for natural hosts and adjacent commercial groves in conjunction with element III.C.4 (Mangan) and IV.A.3 (Moreno). Feasibility of aging methods will be initiated as part of the fluorescent interval marker study (III.C.3.b Moreno) in which natural (pteridine) pigments will be investigated.

IV.A.3.a Moreno, D.

CRIS 6204-43000-003-00D

SY: 0.2

Objective: Determine the suitability of commercial fruits as hosts for the Mexican fruit fly.

Significance: There are about 65 different hosts listed for the Mexican fruit fly. However, it is questionable that every host listed is a suitable host. Of the commercial fruit crops, citrus is listed as the principle commercial host of the Mexican fruit fly. It might be that indeed all citrus varieties are attacked with the same intensity, except that some varieties may allow a higher larval survival of fruit flies. Thus, a more suitable host would allow higher survival. If we can categorize commercial fruits as to their suitability as a host for the Mexican fruit fly, perhaps we can better allocate resources for the fly-free concept, fly detection, fly eradication, monitoring of populations and fly control.

Justification: Industry - III.G; Regulatory - III.NN.

Constraints: The process of going through most of the commercial fruits already listed as hosts can be long and tedious. Preferably only fruit that has not been treated with pesticides should be used. Also, only fruit that is tree ripened should be used. Therefore, one needs to work with the seasonal production of the various fruits. The process becomes expensive because of the length of time that it takes to conduct the study and the time that it takes to locate and transport appropriate fruits.

References: None.

Co-investigators/Cooperators: R. L. Mangan, ARS, Weslaco, Texas; M. Sanchez R, ARS Collaborator, General Teran, Nuevo Leon, Mexico. Potential: APHIS S&T, Mission, Texas.

Work Plans for FY 92: Work out the methodology of infestation to acquire uniformity of infestations. Determine an adequate sampling of infested fruit to estimate the starting population in terms of eggs. Develop a minimal diet to feed adult insects based on the amount of proteins and carbohydrates available in fresh fruits.

Also see Harris IV.A.1.a, Harris IV.A.1.a, Liquido IV.A.7.a.

b. Process

4. Feeding

Also see Landolt I.H, Landolt I.H.

5. Diurnal Rhythm

6. Developmental Modeling

a. Individual

IV.A.6.a Vargas, R.

CRIS 5320-43000-006-00D

SY: 0.1

Objective: Develop temperature driven demographic models for all four species of fruit flies in Hawaii.

Significance: Temperature based demographic models are needed to project species distribution and abundance throughout Hawaii. A Geographic Information System (GIS) would be utilized to map fruit fly distribution and abundance on the basis of temperature, first for the island of Kauai and second for the state of Hawaii. Such information would be useful for determination of projected ranges of fruit flies within the mainland U. S. and provide data on fruit fly reproduction for operational programs.

Justification: Industry - III.G; Regulatory - III.X, III.CC.

Constraints: None.

References:

- Vargas, R. I., D. Miyashita and T. Nishida. 1984. Life history and demographic parameters of three laboratory-reared tephritids (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 77:651-656.
- Vargas, R. I. and J. R. Carey. 1990. Comparative survival and demographic statistics for wild oriental fruit fly, Mediterranean fruit fly, and

melon fly (Diptera: Tephritidae) on papaya. J. Econ. Entomol. 83:1344-1349.

Co-investigators/Cooperators: J. Spencer, ARS, Kapaa; S. Seo and H. Chang, ARS, Honolulu; APHIS S&T, Hawaii Sterile Fruit Fly Facility.

Work Plans for FY 92: Complete temperature studies for laboratory-adapted strains (60, 70, 80, 90°F).

b. Population

IV.A.6.b Vargas, R.

CRIS 5320-43000-006-00D

Also: IV.A.7

SY: 0.1

Objective: Conduct detailed studies of the relationship between fruit infestation and fruit fly trap capture (sampling techniques, population estimates and computer modelling).

Significance: Due to the relative weakness of trimedlure, population estimates of Mediterranean fruit fly are difficult on the basis of trap captures. The proposed study would provide information on the correlation between fruit sampling and trap captures and allow for population estimates on the basis of conventional sampling theory. A Geographic Information System (GIS) would be utilized to estimate populations first on the island of Kauai and second within the state of Hawaii. Such information would be useful for determination of the size of introduced populations within the mainland U. S.

Justification: Regulatory - II.C.

Constraints: None.

References: None.

Co-investigators/Cooperators: J. Spencer, ARS, Kapaa; Akram Kahn, University of Hawaii, Manoa.

Work Plans for FY 92: Complete second year of coffee infestation studies.

IV.A.6.b Sivinski, J.

CRIS 6615-22000-009-00D

SY: 0.2

Objective: Description of Caribbean fruit fly population parameters.

Significance: It is known that different ages and sexes of the Caribbean fruit fly respond to different traps and attractants. The age and reproductive-state composition of fruit fly populations and hence their vulnerability to different monitoring and control measures is unknown. The sources of mortality, life expectancies, developmental periods in different hosts and various other ecological parameters are also undescribed. These are necessary to create realistic models of changes in population growth that could be important to integrated management of fruit flies.

Justification: Regulatory - III.T.

Constraints: None.

References: None.

Co-investigators/Cooperators: R. Baranowski, University of Florida; R. Heath, ARS, Gainesville.

Work Plans for FY 92: Equipment to determine fly age through the accumulation of eye pigments will be obtained and initial laboratory studies performed.

Also see Liquido IV.A.7.a.

7. Population Spatial Models

Also see Harris IV.A.1.a, Harris IV.A.1.a, Harris IV.A.1.a, Mangan IV.A.3.a, Vargas IV.A.6.b.

a. Geographic Information Systems (GIS)

IV.A.7.a *Liquido, N. J.* CRIS 5320-22000-012-00D

Also: IV.A.3.a; IV.A.6.b

SY: 0.2

Objective: Determination of the host range, temporal (seasonal) and spatial (geographic) variations in population density fluctuations of Malaysian fruit fly in Hawaii.

Significance: Although Malaysian fruit fly is known to occur on Oahu, Hawaii since 1983, this pest was detected on other major Hawaiian islands only in 1990. This research will identify the host plants of Malaysian fruit

fly, with particular emphasis on the reservoir host plants in the urban and feral habitats. Furthermore, this research will provide the benchmark data on seasonal abundance and geographic distribution of Malaysian fruit fly in Hawaii. Data gathered from this research will be used in planning the eradication program of this pest in Hawaii and in improving the current detection program in California.

Justification: Regulatory - III.X, III.CCC.

Constraints: None.

References: None.

Co-investigators/Cooperators: E. Harris, ARS, Honolulu; Hawaii Dept. of Agriculture; Univ. of Hawaii; CDFA; APHIS S&T.

Work Plans for FY 92: General survey of Malaysian fruit fly in all islands of Hawaii.

b. Population Estimates

IV.A.7.b Purcell, M. *CRIS 5320-22430-004-00D*

SY: 0.25 (Purcell), 0.1 (Wong)

Objective: Determine quantitative estimates of absolute fruit fly and parasitoid populations in different habitats in Kauai.

Significance: Currently, only relative estimates of fruit fly populations are available (e.g. number of flies per fruit or male flies per trap). While useful in evaluating seasonal population trends and distributional patterns, it would not help derive ratios of parasites released to flies present in a given release area. Estimation of absolute populations would be a invaluable tool for determining the feasibility of various methods of eradicating fruit flies in Kauai.

Justification: Regulatory - III.B, III.J.

Constraints: Access to some areas is limited.

References: None.

Co-investigators/Cooperators: T. Wong, ARS, Honolulu; R. Messing, University of Hawaii, Kauai.

Work Plans for FY 92: Begin studies on the dispersal behavior and population estimates of oriental fruit flies (team effort with Nic Liquido (USDA, Hilo), Russell Messing (Univ. Hawaii), Tim Wong (USDA, Honolulu) and R. Plant (UC, Davis).

Also see Wong III.D.2.c.0.2, Sivinski III.D.2.c.0.4.

8. Mediation by Semiochemicals

IV.A.8 *Light, D.* *CRIS* 5325-22000-010-00D
Jang, E. 5320-22000-005-00D

SY: 0.1 (Light), 0.1 (Jang)

Objective: Study both mate finding and fruit foraging behaviors of Hawaiian fruit flies in the wild and as expressed under laboratory conditions. Study how these behaviors are dependent, interactive and mediated by both pheromonal and environmental chemical ecology.

Investigations of the primary influences on finding and selection of mates by pheromones and host-plants/fruits by kairomones and the potential combined influences of host-plant kairomones on selection of calling/lek sites, production and release of pheromone, and the synergistic interaction with pheromones for attraction and mate selection/courtship. Conversely, the influence of ovipositional deterrent pheromones and allomones on fruit foraging and selection.

Significance: Basic knowledge of the natural expression of the behaviors of mate and host-fruit finding and selection by fruit flies will have fundamental impacts on the design, execution and effectiveness of detection, exclusion and control strategies. Knowledge on the dependence and mediation of these behaviors by semiochemicals will establish a manipulative tool to evolve and optimize our present fruit fly strategies.

Justification: Industry - I.B, III.G, III.H;
 Regulatory - I.D, I.E., I.F, I.J, I.O, II.D, III.O,
 III.V, III.DDD.

Constraints: These detailed studies, especially in the wild, will require much time and patience, and thereby create additional support and travel needs.

References: None.

Co-investigators/Cooperators: N. J. Liquido, ARS, Hilo;
 E. Harris, ARS, Honolulu.

Work Plans for FY 92: Continue laboratory studies on fruit fly behaviors mediated by pheromones, host-plant kairomones and their combinations.

B. Physiology, Biochemistry and Genetics

Background - Very little work has been done in the fields of endocrinology and biochemistry of Tephritidae.

Needs - Studies on the hormonal effects of mating and sperm transfer are needed to understand the factors that control mating drive, remating and sterility.

1. Reproduction and Development

Also see Jang III.A.2.a, Jang III.C.1.a, Jang III.C.1.a.0.1.

a. Ovarian Development

IV.B.1.a Handler, A.

CRIS 6615-22240-001-00D

SY: 0.1

Objective: To identify and characterize yolk polypeptides and the genes which encode. To characterize the developmental and physiological regulation of vitellogenesis in tephritids.

Significance: Central to controlling tephritid populations is understanding the regulation of their reproduction. The synthesis, transport and incorporation of yolk proteins into oocytes, as well as the role of yolk proteins in embryogenesis, are all critical aspects of oogenesis and development. Understanding the basic biology of this system is necessary for the development of biological control agents which can efficiently block it, and for improving laboratory rearing techniques. The diversity in the regulation of vitellogenesis between at least two tephritids, *A. suspensa* and *C. capitata*, indicate that most tephritid species will have to be studied independently.

Justification: Industry - III.D, III.E; Regulatory - III.L, III.M, III.EEE.

Constraints: Physiological studies limited to species allowed in continental U. S.

References:

Handler, A. M. and P. D. Shirk. 1988. Identification and analysis of the major yolk polypeptide from the Caribbean fruit fly, Anastrepha suspensa. Arc. Insect Biochem. Physiol. 9, 91-106.

Co-investigators/Cooperators: None.

Work Plans for FY 92: Isolation and characterization of a genomic clone of the Anastrepha suspensa yolk protein gene will be started. Developmental analysis of yolk protein RNA accumulation will be completed.

2. Endocrinology

Also see Jang I.H.

a. Hormonal

IV.B.2.a Tumlinson, J. CRIS 6615-22000-008-00D

SY: 0.0

Objective: Determine the biosynthetic pathways and mechanisms by which Caribbean fruit flies produce sex pheromonal compounds.

Significance: The Caribbean fruit fly will be the model insect for these studies because of its availability and our knowledge of the constituents of its volatile blends. Eventually we plan to extend these studies to other fruit fly species. These studies are needed to understand the chemical communication systems of fruit flies and to develop effective lures for monitoring and control. Research in Lepidopteran and Dipteran pheromone biosynthesis has repeatedly demonstrated the value of such studies in defining the complete pheromone system of a species. These types of studies will be valuable in developing pheromone-based monitoring and control systems for these important pests.

Justification: Regulatory - I.A, I.D, III.S, III.V.

Constraints: At present all our resources and personnel are committed to other projects. A post doctoral associate and funds for supplies, material and minor items of equipment are needed to make significant progress.

References:

Prestwich, G. D., and G. L. Blomquist. 1987. Pheromone biochemistry. Academic Press, Orlando, FL.

Co-investigators/Cooperators: P. Teal, P. Landolt and C. Calkins, ARS, Gainesville; J. Law and J. Hildebrand, University of Arizona, Tucson.

Work Plans for FY 92: None.

IV.B.2.a Jang. E. B. *CRIS 5320-22000-005-00D*

SY: 0.1

Objective: Determine the role of insect hormones on physiology and behavior of fruit flies.

Significance: Juvenile hormone (JH) and ecdysone are known to influence reproduction, growth and development of insects. These hormones also have a profound influence on fruit fly physiology. Improved knowledge of how hormones influence larval development and adult behavior are needed to understand fruit fly rearing and behavior.

Justification: Industry - III.G; Regulatory - III.PP, III.DDD, III.EEE.

Constraints: None.

References:

Jang, E. B., F. Chang, J. Nagata, and E. Schneider. Effects of benzyl 1,3 benzodioxole on *in vitro* juvenile hormone biosynthesis and release in corpora allata from the Mediterranean fruit fly. (In press).

Co-investigators/Cooperators: D. Light, ARS, Albany,

Work Plans for FY 92: None.

3. Enzymes

Also see Jang III.A.2.a, Jang III.C.1.a, Jang III.C.1.a.0.1.

a. Detoxification

4. Histological

5. Biochemical

Also see Jang III.A.2.a, Jang III.C.1.a.

a. Nutritional

Also see Jang I.H, Jang III.C.1.a.0.1.

b. Biochemical Pathways/Metabolism

IV.B.5.b Jang, E. B. CRIS 5320-22000-005-00D
5320-22000-011-00D

SY: 0.1

Objective: Identify physiological and biochemical vulnerabilities of fruit flies which could be used in exclusion, control and eradication.

Significance: Little is known about how fruit flies deal with physiological stress imposed by chemical, physical or environmental changes. Examining how chemicals such as allelochemicals are metabolized will lead to a better understanding of physiological vulnerability in these species.

Justification: Industry - II.G, III.D, III.G; Regulatory - I.F, II.A, II.D, III.B, III.L.

Constraints: None.

References:

Jang, E. B. Heat shock proteins and thermotolerance in a cultured cell line from the Mediterranean fruit fly, Ceratitidis capitata. (In press).

Co-investigators/Cooperators: None.

Work Plans for FY 92: Develop suitable bioassays to study metabolism of fruit fly cells.

6. Neurophysiology

IV.B.6 Campbell, B. CRIS 5325-22000-010-00D

SY: 0.0

Objective: Isolate and clone the genes which encode the hormonal peptide found in the accessory glands of male medflies which suppresses female receptivity to mating.

Significance: Peptide may be useful in rendering female flies reluctant to mate. Peptide-treated female flies might behave like mated flies and lay only infertile eggs. Future research will make an effort to incorporate the genes encoding these behavior modifying peptides into free-living bacteria for mass-production or transgenic fruit-crops to disrupt fruit fly host or mate searching behaviors. Moreover, fundamental knowledge will be

gained on the structure, expression and function of these genes.

Justification: Industry - III.A, III.C, III.G;
Regulatory - III.B, III.EEE.

Constraints: To construct a cDNA library of male medflies, very fresh flies must be used so that the mRNA is not degraded. There are problems in getting fresh-frozen flies from Hawaii to California. Peptide from male accessory glands must be isolated in order to make a synthetic probe for the cDNA library. A method of introducing the peptide into females in a bioactive form must be developed.

References:

- Munson, et al. Aphid endosymbiosis: evidence of a bacterial infection of an ancestor of 4 aphid families. J. Bacterial. (In press).
Campbell, B. C. and B. M. Unterman. 1989. Purification of DNA from the intracellular symbiotes of Sitophilus oryzae and S. zeamais (Coleoptera:Curculionidae). Insect Biochem. 19:85-88.
Campbell, B. C. 1989. On the role of microbial symbiotes in herbivorous insects. Pp. 1-44, In: Insect-Plant Interactions, Vol. I (E.A. Bernays, ed.) CRC Press Inc. Boca Raton, FL.

Co-investigators/Cooperators: E. Jang, ARS, Hilo; D. Light, ARS, Albany.

Work Plans for FY 92: None.

IV.B.6 Tumlinson, J. CRIS 6615-22000-008-00D

SY: 0.1

Objective: Determine the mechanism of perception of pheromones and other semiochemicals by fruit flies.

Significance: Nothing is known about the biochemical processes that enable fruit flies to detect pheromones and other chemical signals. Aside from electrophysiological studies there have been no investigations of the physiology and biochemistry of pheromone detection and perception in tephritid fruit flies. Studies in Lepidoptera indicate binding proteins and enzymes that metabolize the pheromones are involved in these processes. Knowledge of these processes should lead to the development of methods to interfere with chemical communications in fruit flies. Possible methods include development of compounds that block pheromone perception or that are more powerful attractants than those presently available.

Justification: Regulatory - I.A, I.D.

Constraints: At present, all our resources and personnel are committed to other projects. A post doctoral associate and funds for supplies, material and minor items of equipment are needed to make significant progress.

References:

Prestwich, G. D., G. L. Blomquist. 1987. Pheromone biochemistry. Academic Press, Orlando, FL.

Co-investigators/Cooperators: P. Teal and P. Landolt, ARS, Gainesville; J. Law and J. Hildebrand, Univ. of Arizona.

Work Plans for FY 92: Initiate research on very limited scale to analyze proteins of A. suspensa and the papaya fruit fly.

Also see Jang I.H.

APPENDIX A. ARS RESEARCH ACTION PLAN COMMITTEE

Chairman: C. O. Calkins, Gainesville

Co-chairman: Roy Cunningham, Hilo

Committee Members:

James Coppedge - Beltsville
Robert Faust - Beltsville
Bob Flath - Albany, CA
Barbara Leonhart - Beltsville
Norman Leppla - APHIS, Hyattsville
Bob Mangan - Weslaco
Robert Norton - Beltsville
Richard Soper - Beltsville
Bob Spaide - APHIS, Hyattsville
Ken Vick - Beltsville

Advisors:

Derrell Chambers - APHIS, Guatemala
Patrick Gomes - APHIS, Hyattsville
Al Handler - Gainesville
Bob Heath - Gainesville
Milton Holmes - APHIS, Hyattsville
Eric Jang - Hilo
Richard Parry - Beltsville
Tim Wong - Honolulu

APPENDIX B. NEEDS FOR RESEARCH SUGGESTED BY INDUSTRY

I. Detection and Delimitation:

A. Improvement in lures and traps for Anastrepha species (Ag-Vue, Sunkist, AgriSense, Treas. Fms., CCQC, Cit. Grwrs. Assoc.).
Objectives addressing problem: I.A.2.a Heath, Robacker; I.A.2.b Sivinski, Heath; I.B.5.a Moreno, DeMilo; I.C.1.a Robacker; I.C.2.a Robacker, Heath; I.D.2 Robacker; I.D.4 Robacker; I.G Mayer; III.B.2.c Heath; IV.A.1.a Harris

B. Finding a better medfly lure and trap design (Sunkist, AgriSense, CCQC). Objectives addressing problem: I.A.1 Landolt, Flath, Jang and Liquido, Heath, Light; I.B.1.a Warthen; I.B.1.b DeMilo, Warthen; I.B.1.c Flath, DeMilo; I.B.1.d Cunningham; I.C.2.b Flath and Light, Warthen; I.G Mayer, Warthen; I.H Jang, (Teranishi, Kint and Light); III.B.1.d.0.5 DeMilo; III.B.2.c Heath; IV.A.8 Light and Jang

C. Develop a controlled-release dry formulation for oriental, melon and Malaysian fruit flies (AgriSense). Objectives addressing problem: I.B.2.b Warthen; I.B.7 Leonhardt; I.C.1.a Cunningham; I.C.2.c Jang; I.D.1 Liquido; I.H Liquido and Jang

D. Fruit detection in baggage by a mechanical sniffer (CCQC).
Objectives addressing problem:

II. Exclusion:

A. Development of fumigants to replace EDB and methyl bromide (Cit. Grwrs. Assoc.). Objectives addressing problem: II.A.1.e Armstrong

B. Better host range information for Caribbean fruit fly (Fl. Lime, etc, Brooks). Objectives addressing problem: I.C.2.a Heath; II.A.2 Hennessey and Sharp; II.A.2.a.0.1 Shapiro, Mayer; II.A.2.c Hennessey and Sharp

C. Improved technology and methods to increase efficiency of inspection and exclusion of fruit flies (Am. Farm Bur.).
Objectives addressing problem: I.A.1 Jang and Liquido, I.F.1 Campbell; I.F.4 Thompson; I.G Jang and Light; I.H Jang; II.A.1.b Jang; II.A.1.c.0.2 Mitcham; II.C.2 Calkins; II.C.4 Yokoyama

D. Clearance of carambola cold treatment for Japan (Brooks).
Objectives addressing problem: II.A.1 Armstrong; II.A.1.d Gould

E. Development of a papaya fruit fly quarantine treatment (Brooks). Objectives addressing problem: II.A.1.c.0.1 Hansen,

II.A.1.c.0.2 Mitcham; II.A.2 Hennessey and Sharp; II.A.2.c Hennessey and Sharp; III.C.1.b Hansen

F. Determine the economic and quarantine status of hitchhiking insects (Brooks). **Objectives addressing problem:**

G. Continue research on alternative commodity and pest exclusion treatments (CCQC, CTFA). **Objectives addressing problem:** II.A.1 Armstrong, Hallman; II.A.1.a Hallman; II.A.1.b Jang; II.A.1.c.0.1 Gould, Hallman, Armstrong, Sharp, Hansen, Mangan; II.A.1.c.0.2 Chan, Armstrong, Miller, Mitcham; II.A.1.d Miller, Yokoyama; II.A.1.e Armstrong; II.A.2 Hennessey and Sharp; II.A.2.a.0.1 Shapiro, Mayer; II.A.2.a.0.2 Yokoyama, Greany, McDonald; III.C.1.b Hansen; IV.A.2 Mankin; IV.B.5.b Jang

H. Discover chemical and non-chemical alternatives in meeting quarantine control levels (CCQC, CTFA) **Objectives addressing problem:** II.A.1 Armstrong; II.A.1.b Hallman, Jang; II.A.1.c.0.1 Mangan; II.A.1.c.0.2 Miller, Mitcham; II.A.1.d Miller, Yokoyama; II.A.1.e Armstrong; II.A.2 Hennessey and Sharp; II.A.2.a.0.1 Waiss; II.A.2.a.0.2 Yokoyama, Greany, McDonald; II.A.2.c Hennessey and Sharp; II.B.3 Yokoyama

III. Control and Eradication and Fundamental Biology:

A. Find a substitute for malathion bait sprays (Ag-Vue, Cit. Grwrs. Assoc., Am. Farm Bur., Treas. Farms). **Objectives addressing problem:** I.A.1 Landolt; I.C.1.a Cunningham; I.H Liquido and Jang; III.A.2.a Mangan, Vail; III.A.2.b Moreno; III.A.2.d Vail; III.B.1.d.0.4 Leonhardt; III.B.1.d.0.5 DeMilo; III.B.2.c Heath; IV.A.3.a Mangan; IV.B.6 Campbell

B. Continue Ceralure and male annihilation research (Ag-Vue, AgriSense). **Objectives addressing problem:** I.B.1.b DeMilo, Warthen; I.B.1.d DeMilo; I.H (Teranishi, Kint and Light); III.B.1.d.0.1 Liquido; III.B.1.d.0.5 DeMilo; III.B.2.c Heath

C. Find a substitute for aerial bait sprays (CCQC). **Objectives addressing problem:** I.A.1 Heath; I.A.2.a Heath; I.A.2.b Heath; I.C.1.a Cunningham; I.G Mayer; I.H Liquido and Jang; III.A.2.a Mangan; III.A.2.b Moreno; III.B.1.d.0.5 DeMilo; III.B.2.c Heath; III.D.2.b Schroeder; IV.B.6 Campbell

D. Improve rearing technology for all fruit flies (Ag-Vue, Sunkist, Treas. Farms). **Objectives addressing problem:** III.C.1 Hennessey; III.C.1.a Jang, Chan; III.C.1.a.0.1 Jang; III.C.1.a.0.2 Chan; III.C.1.b.0.1 Moreno; IV.B.1.a Handler; IV.B.5.b Jang

E. Develop biocontrol methods for fruit flies: Formulations to dispense more easily, viruses, bacteria, fungi, protozoa and

nematodes (Ag-Vue, AgriSense, Am. Farm Bur.). **Objectives addressing problem:** I.H Robacker; III.A.2.a Mangan, Vail; III.A.2.d Vail; III.D.1.d Lindegren; III.D.2.b Schroeder; IV.B.1.a Handler

F. Develop technology to mass produce parasites on artificial diet (AgriSense). **Objectives addressing problem:** III.C.1.a.0.1 Jang; III.D.2.e.0.3 Harris

G. More research on medfly, Mexican, Oriental, Caribbean and melon fruit flies (Fl. Lime ...). **Objectives addressing problem:** I.A.1 Heath, Light; I.A.2.a Heath; I.A.2.b Heath; I.B.1.c DeMilo; I.B.1.d Cunningham; I.B.2.b DeMilo, Liquido and Cunningham; I.B.5.a DeMilo; I.C.1.a Cunningham; I.C.2.a Heath; I.F.1 Campbell; I.G Mayer; I.H Liquido and Jang; III.A.2.a Mangan; III.B.1.d.0.1 Liquido; III.B.2.c Heath; III.C.1 Hennessey; III.C.2.b.0.1 Seo; III.C.3.a Moreno, Heath; III.C.3.b Moreno, III.C.4 Mangan; IV.A.1.a Liquido; IV.A.3.a Moreno; IV.A.6.a Vargas; IV.A.8 Light and Jang; IV.B.2.a Jang; IV.B.5.b Jang; IV.B.6 Campbell

H. Improve SIT technology (CCQC, Am. Farm Bur.). **Objectives addressing problem:** I.A.1 Jang and Liquido, Light; I.G Jang and Light; I.H Jang; III.A.2.a Mangan; III.C.1.a Chan and Jang; III.C.1.c.0.1 Handler; III.C.1.d.0.2 Handler; III.C.2.a Seo; III.C.2.b Vargas; III.C.2.b.0.1 Seo; III.C.2.b.0.2 McInnis; III.C.3.a Heath; III.C.3.d Vargas, McInnis; III.C.4 Seo, Mangan; III.D.2.b Schroeder; IV.A.1.a Liquido; IV.A.8 Light and Jang

I. Conduct a survey of economic fruit flies in importing countries (Brooks). **Objectives addressing problem:** I.F.1 Campbell; III.C.4 Mangan; IV.A.3.a Mangan

J. What are the economic consequences of the establishment of an exotic fruit fly in one of the citrus growing states? (Brooks, Cit. Grwrs Assoc.). **Objectives addressing problem:**

AG-VUE, Agricultural Consulting
P. O. Box 4537
Blue Jay, CA 92317

Sunkist Growers, Inc.
P. O. Box 7888
Van Nuys, CA 91409

Citrus Growers Associates, Inc.
2930 Winter Lake Road
Lakeland, FL 33803

Florida Lime and Avocado Admin. Committees
P. O. Box 188
Homestead, FL 33090-0188

American Farm Bureau Federation
225 Touhy Ave.
Park Ridge, IL 60068

AgriSense
4230 West Swift, Suite 106
Fresno, CA 93722

J. R. Brooks and Son, Inc.
P. O. Drawer 9
18400 S.W. 256 St.
Homestead, FL 33090

Treasure Farms
13042 W. Myford Road
Irvine, CA 92720

California Citrus Quality Council
953 West Foothill Boulevard
Claremont, CA 91711

California Tree Fruit Agreement
P. O. Box 255383
Sacramento, CA 95865-5383

APPENDIX C. NEEDS FOR RESEARCH SUGGESTED BY ACTION AND REGULATORY AGENCIES

I. Detection:

A. New detection tools are needed to detect incipient infestations for longer distances using less man power at a cheaper cost. Dry traps are preferred rather than liquid traps using synthetic or natural attractants for Anastrepha (APHIS, TX, FL)*. Objectives addressing problem: I.A.1 Landolt, Flath, Jang and Liquido, Heath; I.A.2.a Heath, Landolt, Robacker; I.A.2.b Sivinski, Heath; I.A.3 Leonhardt; I.B.1.c Flath; I.B.1.d Cunningham; I.B.2.b Liquido and Cunningham; I.B.4.a Flath; I.B.5.a Moreno; I.B.7 Leonhardt; I.C.1.a Robacker; I.C.1.a Cunningham; I.C.2.a Robacker, Heath; I.C.2.b Flath and Light; I.C.2.c Flath and Light; I.D.1 Liquido; I.D.2 Robacker; I.D.4 Robacker; I.G Mayer; I.H Landolt, Jang, Liquido and Jang; III.B.1.c Liquido; III.B.1.d.0.1 Liquido; III.B.2.c Heath; IV.A.1.a Harris; IV.B.2.a Tumlinson; IV.B.6 Tumlinson

B. Clear guidelines on optimal placement of traps to detect infestations at low levels based of fly biology and behavior, preferred hosts, parts of trees, seasonal differences, etc. (APHIS, TX). Objectives addressing problem: I.A.1 Heath; I.A.2.a Heath; I.A.2.b Heath; I.C.2.a Heath; III.B.1.d.0.1 Liquido; III.B.2.c Heath; IV.A.1.a Harris

C. Taxonomic tools are needed to distinguish adult male and larval forms that are detected during trapping and fruit cutting. Tools must be usable to inspectors (APHIS, FL). Objectives addressing problem: I.F.1 Campbell; I.F.4 Thompson, Norrbom, Leonhardt

D. Powerful attractants are needed for male and female fruit flies for surveillance and population suppression (APHIS, LA/ TX, IAEA). Objectives addressing problem: I.A.1 Landolt, Flath, Jang and Liquido, Heath, Light; I.A.2.a Heath, Landolt, Robacker; I.A.2.b Sivinski, Heath; I.B.1.a Warthen; I.B.1.b DeMilo, Warthen; I.B.1.c Flath, DeMilo, I.B.1.d DeMilo, Cunningham; I.B.2.b DeMilo, Liquido and Cunningham, Warthen; I.B.4.a Flath; I.B.5.a Moreno, DeMilo; I.C.1.a Robacker; I.C.1.a Cunningham; I.C.2.a Robacker, Heath; I.C.2.b Flath and Light, Warthen; I.C.2.c Flath and Light, Jang; I.D.4 Robacker; I.G Jang and Light, Mayer, Warthen; I.H Landolt, Jang, Liquido and Jang, (Teranishi, Kint and Light); III.B.1.d.0.1 Liquido; III.B.1.d.0.5 DeMilo; III.B.2.b Landolt; III.B.2.c Heath; IV.A.8 Light and Jang; IV.B.2.a Tumlinson; IV.B.6 Tumlinson

E. Develop population modeling factors for analysis of reduced winter protocols for detection of medflies in various climatic

situations (APHIS). Objectives addressing problem: I.A.1 Light; I.H (Teranishi, Kint and Light); IV.A.8 Light and Jang

F. Alternatives to methyleugenol/naled combination used in detection traps for species of Bactrocera (Dacus) (APHIS, LA). Objectives addressing problem: I.A.1 Light; I.B.2.b Liquido and Cunningham, Warthen; I.G Jang and Light; I.H (Teranishi, Kint and Light); III.A.2.a Jang; III.B.1.d.0.4 Leonhardt; IV.A.8 Light and Jang; IV.B.5.b Jang

G. Develop technique for synthesis of alpha-Copaene and analogs (APHIS). Objectives addressing problem: I.B.1.c DeMilo; I.G Warthen

H. Identify factors that account for relative effectiveness of McPhail and Jackson traps for capture of medflies at certain times of the year (APHIS, LA). Objectives addressing problem: I.A.1 Heath; I.D.1 Liquido; I.H Jang; III.B.2.c Heath; IV.A.1.a Harris

I. Improve formulations of attractants and develop a uniform system of dispensing them such as polymer plugs (APHIS, FL). Objectives addressing problem: I.B.2.b Liquido and Cunningham; I.B.7 Leonhardt; I.C.1.a Robacker; I.C.2.a Robacker; I.D.4 Robacker; I.G Jang and Light, Mayer; III.B.1.d.0.1 Liquido; III.B.1.d.0.4 Leonhardt

J. Investigate attractant/pumpkin volatiles trap for melon fly (CDFA). Objectives addressing problem: I.H Jang; IV.A.8 Light and Jang

K. Evaluate citrus oil extracts combined with Nulure as an Anastrepha attractant (CDFA). Objectives addressing problem: I.C.2.a Heath; I.G Mayer

L. Determine performance of McPhail traps baited with Nulure or Staley's bait under California conditions (L.A. County). Objectives addressing problem:

M. Establish the range of attractancy for trimedlure, ceralure, cuelure, methyleugenol, torula yeast, Pib-7, etc. (FL). Objectives addressing problem: I.B.1.a Warthen; I.B.1.b Warthen; I.C.2.a Heath; I.D.1 Liquido; I.G Mayer; III.B.1.a Purcell; III.B.2.c Heath; IV.A.1.a Liquido

N. Assess the relationship between the density of McPhail traps and the probability of detection (TX) Objectives addressing problem: I.D.1 Liquido

O. Determine the relative attractancy of bait vs. natural chemicals such as plant volatiles, bacteria and bird feces (IAEA). Objectives addressing problem: I.C.1.a Robacker, Cunningham;

I.C.2.a Robacker, Heath; I.C.2.c Jang, I.D.4 Robacker; I.G Mayer;
I.H Robacker, Jang, Liquido and Jang, (Teranishi, Kint and Light);
IV.A.8 Light and Jang

II. Exclusion:

A. Additional commodity treatments are needed for many tropical and sub-tropical fruits (APHIS). **Objectives addressing problem:** II.A.1 Armstrong, Hallman; II.A.1.a Hallman; II.A.1.b Jang; II.A.1.c.0.1 Gould, Hallman, Armstrong, Sharp, Hansen, Mangan; II.A.1.c.0.2 Chan, Armstrong, Miller, Mitcham; II.A.1.d Miller, Gould; II.A.1.e Armstrong; II.A.2 Hennessey and Sharp; II.A.2.a.0.2 McDonald; II.A.2.c Hennessey and Sharp, Armstrong; III.C.1.b Hansen; IV.B.5.b Jang

B. A systems approach is needed for fruit fly suppression and management to expand fly-free zones in the U. S. and Latin America (APHIS). **Objectives addressing problem:** II.A.1.d Gould; II.A.2 Hennessey and Sharp; II.A.2.c Hennessey and Sharp; III.B.1.a Vargas; III.C.4 Mangan; III.D.1.d Lindegren; III.D.2.c.0.4 Calkins

C. Statistical sampling procedures are needed for fruit cutting at quarantine road station, packing sheds and market inspections (APHIS). **Objectives addressing problem:** II.C.4 Yokoyama; IV.A.6.b Vargas

D. There is a need to verify host susceptibility to fruit fly attack (APHIS). **Objectives addressing problem:** II.A.2 Hennessey and Sharp; II.A.2.a.0.1 Shapiro, Greany, Mayer; II.A.2.a.0.2 Yokoyama; II.A.2.c Hennessey and Sharp, Armstrong; IV.A.8 Light and Jang; IV.B.5.b Jang

E. New commodity treatments such as new fumigants, use of irradiation, controlled atmospheres and other forms of storage, temperature and physical treatments are needed. Alternative treatments should simplify monitoring, minimize manpower to oversee application and certification and maximize protection of safety and health (APHIS). **Objectives addressing problem:** II.A.1.b Hallman, Jang; II.A.1.c.0.1 Gould, Armstrong, Mangan; II.A.1.c.0.2 Chan, Armstrong, Miller, Mitcham; II.A.1.d Miller, Gould, Yokoyama; II.A.1.e Armstrong; II.A.2.a.0.1 Weiss; II.A.2.a.0.2 McDonald

F. Determination of which fruit can be certified while immature (green) (APHIS). **Objectives addressing problem:** II.A.2 Hennessey and Sharp; II.A.2.a.0.1 Shapiro, Greany, Mayer; II.A.2.c Hennessey and Sharp, Armstrong

III. Control/Eradication and Fundamental Biology:

A. Substitutes for aerially applied malathion bait spray that are environmentally and publicly acceptable. Evaluate potential of Bt, avermectin B and viruses as a substitute for malathion (APHIS, CDFA, IAEA). **Objectives addressing problem:** III.A.2.a Mangan, Jang, Krasnoff, Vail; III.A.2.b Moreno; III.A.2.d Vail; III.B.2.b Landolt; III.B.2.c Heath

B. Biologically-based controls must be developed and integrated with other control methods (APHIS). **Objectives addressing problem:** I.G Mayer; I.H Robacker; III.A.2.a Mangan, Jang, Vail; III.A.2.d Vail; III.B.2.c Heath; III.C.4 Mangan; III.D.1.d Lindegren; III.D.2.b Schroeder, Wong; III.D.2.b.0.1 Calkins; III.D.2.c Purcell; III.D.2.c.0.2 Wong; III.D.2.c.0.4 Calkins; III.D.2.e.0.3 Harris; IV.A.2 Mankin; IV.A.7.b Purcell; IV.B.5.b Jang; IV.B.6 Campbell

C. A substitute is needed for malathion wettable powder for use in ground sprays. Optimal techniques for efficient application such as the ratio of bait to insecticide and the amount of coverage needed (APHIS, CDFA, LA). **Objectives addressing problem:** III.A.2.a Jang

D. There is a need for a less caustic replacement for nu-lure (APHIS, LA). **Objectives addressing problem:** I.A.2.a Robacker; I.C.1.a Robacker, Cunningham; I.C.2.a Robacker; I.D.4 Robacker; I.H Liquido and Jang, (Teranishi, Kint and Light); III.B.2.c Heath; III.C.1.a Chan and Jang

E. Improved soil treatments needed to control larvae and pupae as a replacement for diazinon (APHIS). **Objectives addressing problem:** III.A.1 Purcell; III.A.2.a Jang; III.D.1.d Lindegren

F. Male annihilation technology is needed for the Malaysian and other fruit flies. Develop male annihilation technology for medfly using Ceralure. Formulations that do not use a toxicant are preferred (APHIS). **Objectives addressing problem:** I.B.1.b DeMilo, Warthen; I.B.2.b Liquido and Cunningham; III.B.1.c Liquido; III.B.1.d.0.1 Liquido; III.B.2.c Heath

G. Obverse approach to SIT using sterile female melon flies as "sperm sops" needs to be demonstrated as a potential eradication tool. Compatibility of this approach with simultaneous applications of malathion Min-U-Gel formulation could shorten eradication efforts significantly (APHIS). **Objectives addressing problem:** III.C.2.a Seo

H. Conduct supporting research for registration of chemicals used in male annihilation techniques for each species of fruit flies

(APHIS). Objectives addressing problem: I.B.1.b DeMilo;
III.B.1.a Vargas; III.C.4 Spencer

I. Determine efficacy of boric acid/borax (boron) bait sprays for fruit flies. If warranted conduct initial toxicity studies for boron bait sprays (APHIS). Objectives addressing problem:
III.B.2.c Heath

J. Development of biological control by parasites are needed. Methods for rearing, packing, transport and release, and quality control must be developed on an operational scale to be effective as potential suppression or eradication tactics (APHIS, CDFA, FL). Objectives addressing problem: III.D.2.b Schroeder, Wong;
III.D.2.b.0.1 Calkins; III.D.2.c Purcell; III.D.2.c.0.2 Wong;
III.D.2.c.0.4 Sivinski, Calkins; III.D.2.e.0.3 Harris; IV.A.7.b Purcell

K. Identification of the most effective parasites or complex of parasites to suppress and maintain fruit fly populations at low levels (APHIS). Objectives addressing problem: III.D.2.a Sivinski; III.D.2.b Schroeder; III.D.2.b.0.1 Calkins;
III.D.2.e.0.3 Harris

L. Stable mass production of fruit flies is needed to ensure adequate quantity and quality of flies for control and eradication programs. This requires a well defined diet based on the bionutritional needs and more precise contract specifications for procurement of diet ingredients (APHIS, IAEA). Objectives addressing problem: III.C.1 Hennessey; III.C.1.a Jang, Chan;
III.C.1.a.0.1 Jang; III.C.1.a.0.2 Chan; III.C.1.a.0.4 Calkins;
III.C.1.c.0.3 McInnis; IV.B.1.a Handler; IV.B.5.b Jang

M. Development of mass rearing methods, including development of artificial diets and eggling technologies for other species of Bactrocera and Anastrepha with priority given to B. latifrons and A. obliqua (APHIS). Objectives addressing problem: III.C.1 Hennessey; III.C.1.a Chan and Jang; III.C.1.b.0.1 Moreno; IV.B.1.a Handler

N. Need to determine if chilling affects the pheromone production of medfly and the Mexican fruit fly. Are there short or long term detrimental effects of chilling and/or storing sterile flies (APHIS). Objectives addressing problem: I.A.1 Light; I.H (Teranishi, Kint and Light); III.C.2.a Seo; III.C.3.a Heath

O. Quality control standards for the medfly, Mexican fruit fly, Oriental fruit fly and Bactrocera latifrons requires verification and expansion. New standards are needed to assess effects of packing, shipping, handling and release. Highest priority should be given to B. latifrons (APHIS, CDFA, FL). Objectives addressing problem: I.A.1 Light; III.C.1.a Chan and Jang; III.C.1.a.0.4

Calkins; III.C.1.c.0.1 McInnis; III.C.1.c.0.3 McInnis; III.C.3.a Moreno, Heath; III.C.4 Spencer; IV.A.8 Light and Jang

P. Determine aspects of short and long distance dispersal rates and effect of wind on spread of fruit flies (TX, IAEA). **Objectives addressing problem:** III.C.4 Spencer, Mangan; IV.A.1.a Liquido; IV.A.3.a Mangan

Q. Rearing facilities require greater efficiency at lower costs without sacrificing insect quality or quantity. New methods (recycling spent diet, larval collections, modified eggling cages) or cheaper substitutes for standard diet ingredients must be continually tested prior to making operational changes (APHIS, IAEA). **Objectives addressing problem:** III.C.1 Hennessey; III.C.1.a Jang, Chan; III.C.1.a.0.1 Jang; III.C.1.a.0.2 Chan

R. An improved method of marking adults is needed to facilitate rapid screening. Marker must be nontransferable, have no adverse effects and be conducive to automated ID procedures. Accuracy must be ca. 99% (APHIS). **Objectives addressing problem:** III.C.1 Hennessey; III.C.1.d.0.2 Handler; III.C.3.b Moreno

S. Quality control procedures and standards must be developed for field-released flies. Correlate laboratory tests with field effectiveness (APHIS, FL, LA, TX). **Objectives addressing problem:** III.C.1.a.0.4 Calkins; III.C.1.c.0.1 McInnis; III.C.1.c.0.3 McInnis; III.C.2.a Seo; III.C.2.b Vargas; III.C.2.b.0.1 Seo; III.C.4 Spencer, Mangan; IV.A.2 Sivinski; IV.B.2.a Tumlinson

T. Development of predictive models to improve SIT and detection capabilities based on distribution trends of sterile and wild flies (APHIS). **Objectives addressing problem:** III.C.3.d Vargas; III.C.4 Spencer, Mangan; IV.A.1.a Liquido; IV.A.2 Sivinski; IV.A.3.a Mangan; IV.A.6.b Sivinski

U. How long are released flies effective in the field? How frequent must flies be released to maintain 100:1 ratio. How many flies must be released per square mile (CDFA, LA, TX). **Objectives addressing problem:** III.C.1.a.0.4 Calkins; III.C.3.d Vargas; III.C.4 Spencer, Mangan; IV.A.1.a Liquido

V. Are reared flies from different laboratories compatible with the target wild fly population? (APHIS). **Objectives addressing problem:** I.A.1 Light; III.C.1.a.0.4 Calkins; III.C.1.c.0.1 McInnis; III.C.2.a Seo; III.C.3.a Heath; III.C.4 Mangan; IV.A.8 Light and Jang; IV.B.2.a Tumlinson

W. Is a sterile release of males only better than sterile males and females released together? (APHIS, LA). **Objectives addressing problem:** III.C.3.d McInnis; III.C.4 Spencer

X. Improved predictive models to determine generation times under variable climatic conditions. Determination of other factors that affect the population dynamics of fruit flies are needed to decide program decisions (APHIS). Objectives addressing problem: II.A.1.d Gould; IV.A.6.a Vargas; IV.A.7.a Liquido

Y. Monitoring procedures are needed to assess environmental impacts on certain non-target organisms must be developed concurrently with any new control methods. Control programs need monitoring procedures for existing suppression technologies to satisfy new and changing environmental requirements (APHIS). Objectives addressing problem: III.A.2.a Mangan

Z. Any new control methods must include an assessment of potential adverse effects on non-target organisms, endangered and threatened species (APHIS). Objectives addressing problem: III.D.1.d Lindegren

AA. Predictive models of control alternatives that evaluate potential environmental impacts, such as drift, water run-off, etc. need to be developed (APHIS). Objectives addressing problem:

BB. Continue development of DNA and isozyme analysis research projects for identifying and distinguishing medfly and other tephritid species (APHIS). Objectives addressing problem: I.F.1 Campbell

CC. Construct climatological models for the exotic economic fruit flies for the United States (APHIS, CDFA). Objectives addressing problem: II.A.1.d Gould; IV.A.6.a Vargas

DD. Necessity of covering commercial host fruit at fruit stands and markets within quarantine areas for regulatory purposes (FL DPI). Objectives addressing problem:

EE. Need to bioengineer a strain of Bt. to be strain specific to fruit flies (CDFA). Objectives addressing problem: I.F.1 McInnis, Huettel, Sheppard

FF. Evaluate the impact of roving vs. aerial release of adults for survivability and post-release behavior of medfly and Mexican fruit fly (CDFA). Objectives addressing problem: III.B.1.a Vargas; III.C.2.a Seo; III.C.3.d Vargas; III.C.4 Spencer, Mangan

GG. Investigate new trap design efficiencies as they relate to medfly overflooding ratios and Dacus species (CDFA). Objectives addressing problem: I.D.1 Liquido; III.C.4 Spencer

HH. Evaluate foliage treatment only as a replacement for soil drench (CDFA). Objectives addressing problem: III.A.2.a Mangan

- II. Provide a replacement for DDVP in Steiner traps (CDFA).
Objectives addressing problem: I.D.1 Liquido; III.A.2.a Jang
- JJ. Biology studies on B. zonatus including hosts, overwintering (CDFA). **Objectives addressing problem:**
- KK. Determine if trimedlure or Ceralure show any seasonality due to changes in physiology of the fly (CDFA). **Objectives addressing problem:**
- LL. Disposal of borax and insecticide impregnated wicks (CDFA).
Objectives addressing problem:
- MM. Develop methodology to directly assess the impact on the reproductive success of the target population (CDFA). **Objectives addressing problem:** III.C.4 Mangan
- NN. Identify tephritids and their hosts from all foreign countries and identify introduction pathways (CDFA). **Objectives addressing problem:** I.F.1 Campbell; I.F.4 Norrbom; IV.A.3.a Moreno
- OO. Test reliability and constraints of using SIT as the sole eradication method for Anastrepha species (CDFA). **Objectives addressing problem:** III.C.4 Mangan; IV.A.3.a Mangan
- PP. Develop field and laboratory studies to assess the bioclimatic potential of economic species (CDFA). **Objectives addressing problem:** II.A.1.d Gould; IV.A.2 Mankin; IV.B.2.a Jang
- QQ. Bait spray: frequency, duration, rain effects (LA) (CDFA).
Objectives addressing problem: I.C.1.a Cunningham; I.H Liquido and Jang; III.B.2.c Heath
- RR. Decision to use either air or ground spray: number flies, number of larvae, size of the area to be sprayed (LA). **Objectives addressing problem:**
- SS. Efficacy of aerial vs. ground release of flies, including backup that would be needed if the planes were grounded (LA).
Objectives addressing problem: III.C.3.d Vargas; III.C.4 Mangan
- TT. Possibility of shipping eggs to a rearing center where larvae would be reared and sterilized pupae would be shipped out. No adult flies would be allowed to exist (LA). **Objectives addressing problem:**
- UU. Reduce or eliminate the threat of Mexican fruit fly reinfestations from Mexico (TX). **Objectives addressing problem:** I.B.5.a DeMilo; III.C.4 Mangan; IV.A.3.a Mangan

VV. Demonstrating efficacy of SIT under field conditions (TX).
Objectives addressing problem: III.C.2.b.0.2 McInnis; III.C.3.d Vargas

WW. Determine the number of sterile flies necessary to overflow a wild population (TX) **Objectives addressing problem:**
 III.C.1.a.0.4 Calkins; III.C.2.a Seo; III.C.3.d Vargas; III.C.4 Seo, Mangan

XX. Reproductive physiology of fertile and sterile flies: number of sperm produced, number and timing of rematings of fertile females, etc. (TX). **Objectives addressing problem:** I.C.2.c Jang; III.C.1.c.0.1 Handler; III.C.3.a Heath; III.C.4 Mangan

YY. Improve genetic sexing strains in medfly and develop comparable strains in other important strains (IAEA). **Objectives addressing problem:** III.C.1.c.0.1 McInnis, Handler

ZZ. Develop methods to genetically transform the germlines of fruit flies by developing a transformation vector and a suitable marker (IAEA). **Objectives addressing problem:** III.C.1.c.0.1 McInnis; III.C.1.d.0.2 Handler

AAA. Erect molecular maps of the genomes of fruit flies with RFLPs (IAEA). **Objectives addressing problem:** I.F.1 Campbell; I.F.4 Campbell

BBB. Determine the genetics of traits that would be useful in strains to be released in SIT programs, e.g. those that would facilitate storage and stockpiling, improved searching behavior longevity and ease of identification (IAEA). **Objectives addressing problem:** III.C.1.c.0.1 McInnis

CCC. Develop user-friendly software programs to facilitate collection and management of rearing, quality control and field data (IAEA). **Objectives addressing problem:** IV.A.7.a Liquido

DDD. Study foraging behavior in relation to physiological state, sensitivity of resources, etc. (IAEA). **Objectives addressing problem:** I.C.2.c Jang; I.H Robacker, Jang, (Teranishi, Kint and Light); IV.A.3.a Mangan; IV.A.8 Light and Jang; IV.B.2.a Jang

EEE. Investigate the endocrine system to determine the role of hormones and neuropeptides on pheromone production, dispersal, remating behavior, etc. (IAEA). **Objectives addressing problem:** I.H Jang; II.A.2.a.0.1 Shapiro, Mayer; IV.B.1.a Handler; IV.B.2.a Jang; IV.B.6 Campbell

FFF. Develop effective IPM programs for population suppression of fruit fly complexes in importing countries (IAEA). **Objectives**

addressing problem: III.A.2.a Mangan; III.D.1.d Lindegren;
IV.A.3.a Mangan

GGG. Develop substitutes for cuelure for male annihilation systems against melon fly which will attract sexually immature flies (CDFA, APHIS). **Objectives addressing problem:**

* - APHIS = USDA-APHIS; CDFA = California Dept. Food and Agriculture; FL = Florida Div. Plant Industry; IAEA = International Atomic Agency, Vienna; L.A. = Los Angeles County; TX = Texas Dept. of Agriculture.

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APPENDIX H. GAPS IN THE FRUIT FLY RESEARCH ACTION PLAN

Detection:

1. Develop a dry trap for liquid food baits.
2. Develop food lures for Anastrepha species.
3. Isolate, identify and synthesize sex pheromones for the economic species of Anastrepha.
4. Determine the range of attractancy for existing lures and for new formulations of existing lures. Determine the trap density needed to detect small populations of flies.
5. Develop methods of detection of fruit in baggage, i.e., a mechanical sniffer.
6. Increase basic biological and behavioral studies in the field of the economic species of fruit flies.

Exclusion:

1. Identify research teams to review and devise new of innovative technologies for interception of agricultural commodities/exotic insects.
2. Exclusion group scientists meet with APHIS, industry to establish national priorities for commodity treatment research. Identify resources and assign responsibilities.
3. Initiate research programs to statistically evaluate probabilities of exotic pest interception, survival and establishment.
4. Expand research to determine causes of host use by various fruit fly species.
5. Review statistical procedures for quarantine security alternatives to "Probit 9" without loss of level of security.
6. Integrate quarantine treatments to include phytotoxic effects, overall quality, shelf life, marketing, general harvest, handling and packing procedures.

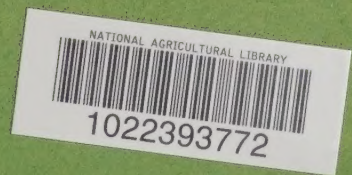
Control/Eradication:

1. Develop rearing procedures for the South American fruit fly, Anastrepha fraterculus.
2. Initiate exploration for fruit fly parasites in Latin America, Africa and Southeast Asia.
3. Find a replacement for malathion and diazinone used in bait sprays and soil drenches, respectively.
4. Reduce the amount of malathion applied per acre in bait sprays.
5. Develop quality evaluations for flies that have been released in the field.
6. Develop packaging and shipping procedures for Anastrepha fruit flies.
7. Reduce emphasis on melon fly annihilation tests.

APPENDIX I. PRIORITY NEEDS OF MEDFLY RESEARCH IDENTIFIED
AT THE CDFA WORKSHOP, OCTOBER 16 AND 17, 1991

1. Improve lure efficiency
2. Identify new lures
3. Improve behavioral response to attractants
4. Improve trap efficiency:
 - a. Trap types
 - b. Optimal site location for traps (hosts, etc.)
 - c. Trap location (posting vs. sweeping)
5. Identify new traps
6. Basic biology of small populations:
 - a. Reproductive biology/dynamics of sexual selection
 - b. Invasion biology
7. Population identification:
 - a. Genetic or biochemical population markers
 - b. Larval age
 - c. Gut contents
8. Larval detection - acoustical methods
9. Artificial sniffer for inspection/detection of baggage
10. Develop operational profile for fruit smugglers
11. Develop educational programs for travellers
12. Determine primary pathways of entry - commercial vs. airports
13. Behavior and biology in native home (Africa)
14. Improve attracticides (attractants and delivery systems for eradication)
15. New lures for male annihilation
16. Malathion research to reduce dosage and create innovative uses
17. Identification and uses of biological insecticides:
 - a. *Bacillus thuringiensis* (new strains)
 - b. Viruses
 - c. Nematodes
 - d. Genetically enhanced strains
18. Introduce natural enemies in areas of high populations to reduce pressure for introductions
19. Augmentative releases of natural enemies in combination with other tactics
20. Economic analysis of inundative/augmentative releases
21. Elucidate and improve criteria for declaring eradication:
 - a. Detection criteria
 - b. Length of time
22. Sterile insect technique:
 - a. Refinement of release rates
 - b. Produce better quality insects
 - c. Compatibility with wild flies
 - d. Competitiveness of males in the lek
 - e. Efficacy of single sex vs. both sexes released
 - f. Basic studies of the physiology of fertility to develop new agents for sterilization

- 23. Basic biology of small fly populations in relation to dynamics of the sexual selection process
- 24. Economics of medfly establishment in California
- 25. Reevaluation of medfly host range





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